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## STUDIES ON BOVINE MASTITIS.

### IX.—A SELECTIVE MEDIUM FOR THE DIAGNOSIS OF STREPTOCOCCUS MASTITIS.

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IN the bacteriological examination of milk samples for the presence of micro-organisms associated with chronic mastitis, a medium of choice would be one which favoured their growth and which either inhibited saprophytic bacteria or rendered their colonies easily distinguishable. Saprophytic bacteria occur normally in the udder, or may gain access to the milk from outside during the taking of a sample. Bacteria from sources outside the udder could, of course, be excluded if very special precautions were taken during the collection of samples, but under practical conditions quite simple measures have to be adopted to avoid this source of contamination.

The need for a selective or differential medium has been realised by those who have investigated cultural methods for the diagnosis of bovine mastitis, since milk at times contains a host of saprophytic bacteria which may cause confusion.

An account is given in this paper of a medium which has proved of great service in the examination of large numbers of milk samples for the presence of mastitis streptococci.

Cultural methods of selective value for the isolation of mastitis streptococci have been devised previously. Thus, Klimmer, Haupt and Roots (1928) made extensive use of a special agar medium containing saccharose and brom-cresol-purple. On the surface of this medium they state that colonies of *Str. agalactiæ* are surrounded by yellow zones, whereas the milk contaminant, *Str. lactis*, usually does not change the colour of the medium. Seelemann (1928) recommended surface cultivation on agar containing sodium nucleinate for the recognition of colonies of mastitis streptococci.

Diernhofer (1932), while recognising the value of the brom-cresol-purple medium, points out that many strains of *Str. lactis* as well as many other saprophytic bacteria ferment saccharose and so produce yellow colonies. With regard to the appearance of surface colonies on

agar plates, it was found by Bischoff (1930) and by Diernhofer (1932, 1933) that colonies of the common mastitis streptococcus do not always present the typical "rough" appearance described by Seelemann. Diernhofer prefers surface cultivation on glucose blood agar, a medium which favours the growth of streptococci.

In earlier work at this Institute (Minett, Stableforth and Edwards, 1930) use was made of a cultural method involving deep cultivation of milk deposits in agar plates containing 5 per cent. ox blood. This medium is of great value for the recognition of beta-hæmolytic streptococcus colonies, but it has certain disadvantages. Firstly, colonies of staphylococci, *Bact. coli*, and of surface-spreading organisms such as *B. subtilis* are sometimes present in such large numbers as to obscure the growth of streptococcus colonies. Secondly, it is not always easy to distinguish isolated colonies of non-hæmolytic streptococci from those of staphylococci, so that an exhaustive verification of colonies by picking into broth may be necessary. Under these conditions, it is possible that cows harbouring small numbers of streptococci in the udder might be missed.

### The Use of Crystal Violet for Selective Media.

It was shown by Churchman (1912), and by Krumwiede and Pratt (1914), that certain streptococcus strains were more resistant to the bactericidal action of aniline dyes than other Gram-positive organisms. Haxthausen (1927) devised a selective medium for the isolation of streptococci from skin by adding crystal violet (hexa-methyl-pararosaniline) to glucose broth. Bryan (1932) found that by adding to blood agar a closely related compound, gentian violet, it was possible to prevent the growth of saprophytic bacteria commonly found in milk, while that of streptococci was unaffected.

A. *A Liquid Medium.*—Details of a liquid selective medium have already been published (S. J. Edwards, 1930).

Briefly stated, this medium consisted of meat infusion broth (*p*H 7·4) to which was added glucose and serum in concentrations of 1 per cent. and 5 per cent. respectively. In tubes measuring 13 by 1·3 cm. filled with liquid to a height of about 6 cm., small numbers of streptococci grew profusely. The addition of crystal violet* to this medium to a concentration of 1 : 200,000 completely inhibited the growth of the most common milk saprophytic organisms, *viz.*, staphylococci and *B. subtilis*, while that of streptococci was unhindered.

For the examination of milk samples this liquid medium was used either alone or in conjunction with blood agar plates. The centrifuged deposits obtained from 10 c.c. of milk were sown into tubes of liquid medium, and, if their amount was abnormal, *i.e.*, 0·01 c.c. or over, a further cultural examination was made in blood agar. After incubation for 24 hours any growths present were examined microscopically, and if streptococci were found their nature was determined by plating in blood agar and by transferring single colonies to test media. It was possible in this way to examine a large number of milk samples expeditiously since it was a common experience that many tubes remained sterile.

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* A product from G. T. Gurr, 136, New King's Road, London, S.W.1.

The use of the liquid medium can be recommended in the re-examination of the milk of healthy cows which are being periodically tested in order to determine whether their udders are still free from streptococcus infection. When a herd is being examined for the first time, however, and probably contains a fair proportion of infected cows, considerable labour and delay are involved in carrying out the subsequent plating in blood agar of growths found to consist of streptococci.

B. *A Blood Agar Medium.*—In view of the limited value of the liquid medium in practice, it was considered advisable to adhere to deep cultivation of milk samples in blood agar plates and to ascertain by experiment whether, by the use of crystal violet, it was possible to inhibit the growth of contaminating organisms which ordinarily render the detection of streptococcus colonies difficult. For this purpose, a number of streptococcus and contaminant strains were sown into blood agar containing various concentrations of crystal violet, and after incubation at 37° C. growth in the dye-containing medium was compared with that in plain blood agar.

TABLE I.

THE EFFECT OF CRYSTAL VIOLET IN DIFFERENT CONCENTRATIONS ON THE GROWTH OF BETA-HÆMOLYTIC STREPTOCOCCI, STAPHYLOCOCCI AND *B. subtilis*.

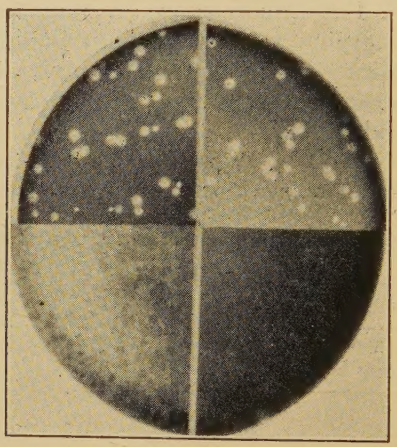
| *Organism.* | *Plain Blood Agar (Control).* | *Crystal Violet Blood Agar.* | | |
|---|---|---|---|---|
| | | 1 : 400,000 | 1 : 200,000. | 1 : 100,000. |
| *Strep.* 1. Dil. 1 | 500 cols. narrow beta zone | as control | as control | as control* |
| ,, 2 | 15 | 18 | 12 | 12 |
| *Strep.* 2. Dil. 1 | 500 cols. narrow beta zone | as control | as control | as control* |
| ,, 2 | 10 | 13 | 22 | 13 |
| *Strep.* 3. Dil. 1 | 600 cols. narrow beta zone | as control | as control | as control* |
| ,, 2 | 55 | 45 | 60 | 60 |
| *Staph.* 1. | 800 gamma cols. | O | O | O |
| 2. | 1,000 beta cols. | O | O | O |
| 3. | Innum. do. | O | O | O |
| *B. subtilis* | do. | O | O | O |

*With 1 : 100,000 concentration, colonies were just visible and beta-hæmolytic zones very narrow.
O = no growth.

Table I illustrates the results of one experiment in which dilutions of three different beta-hæmolytic streptococcus cultures were made in saline, and equal amounts (one drop from a Pasteur pipette) plated in

meat extract agar (*p*H 7·5) containing 5 per cent. ox blood, as well as in the same medium containing three different concentrations of crystal violet. At the same time, saline dilutions of different staphylococcus and *B. subtilis* cultures were made, and equal amounts (one drop, usually containing 1,000 organisms) sown into similar media. After 48 hours' incubation at 37° C. the numbers and size of the colonies in the dye plates were compared with those in plain blood agar.

The results show that, while dye concentrations of 1 : 400,000 and 1 :200,000 had no influence on the growth of the streptococcus strains, a concentration of 1 :100,000 definitely reduced the size of the individual colonies and of the surrounding zones of hæmolysis. On the other hand, the growth of the staphylococcus and *B. subtilis* strains was completely inhibited at all concentrations of dye.



*a.* *b.*

FIG. 1.

(*a*) Plain blood agar.
(*b*) The same with 1 : 500,000 crystal violet.
The growth of *B. subtilis* present in (*a*) has been completely inhibited in (*b*), while the growth of the beta-hæmolytic streptococcus is similar in both cases.

In examining the behaviour of numerous beta-hæmolytic and non-hæmolytic streptococcus strains in blood agar containing progressively increasing concentrations of crystal violet, it was observed that the primary toxic effect of the dye on the growth of streptococci was to diminish the size of colony, and, in the case of beta-hæmolytic strains, the size of the hæmolysed zones. Within the limits of technical error, however, the actual numbers of colonies were not reduced. Consequently, the development of beta-hæmolytic streptococci has been taken as a basis in establishing a suitable dye concentration.

Now, samples of crystal violet supplied by different manufacturers differ widely in their bactericidal action, mainly owing to variations in the amount of impurities they contain. In order

to obtain consistent results with crystal violet, therefore, it was necessary to use a product of standard potency. A dye prepared by British Drug Houses (B.D.H.), Ltd., Graham Street, London, N.1, is now in use which contains 88·5 per cent. of hexa-methyl-pararosaniline, and, according to the makers, this is the purest form that can be prepared. Tests with this product show that the best results are given by a final concentration of 1 : 500,000, since, at this dilution, the development of beta-hæmolytic streptococci is not affected, while bactericidal action on staphylococci and *B. subtilis* is at its maximum (Fig. 1). A stock solution of the purified dye may be made by dissolving 0·1 gm. in 100·0 c.c. of distilled water to which one or two drops of strong hydrochloric acid are added to prevent the formation during storage of an insoluble basic hydrochloride.

*The Differentiation of Mastitis Streptococci from other Dye-resistant Organisms.*

The use of crystal violet considerably reduces the labour of reading blood agar plates by preventing the growth of predominating milk contaminants. Unfortunately, however, organisms occur quite frequently in milk, such as *Bact. coli*, *Str. lactis* and a variety of staphylococcus, which are not inhibited by the dye. Attempts, therefore, were made to incorporate in the dye medium substances which would either prevent the growth of these organisms or enable a distinction to be drawn between their colonies and those of streptococci.

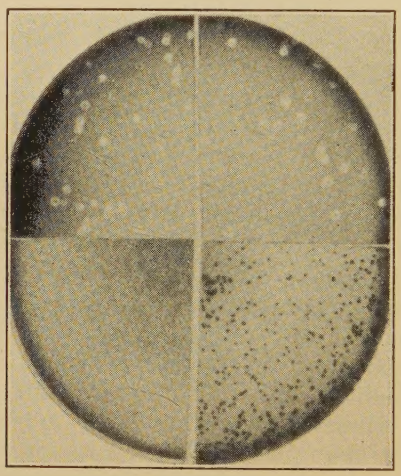
In the first place, potassium tellurite was tried with the object of inhibiting *Bact. coli*. This substance, however, caused hæmolysis in blood agar in concentrations as low as 1 :50,000, and, in a liquid medium without blood and containing still higher concentrations of tellurite, only a few *Bact. coli* strains from milk were sensitive. By using a substance, such as mannite, which is fermented only by the dye-resistant organisms, it was thought that their colonies could be distinguished when a suitable indicator was also present. With brom-cresol-purple, however, the colour produced was insufficient to allow of a satisfactory differentiation in blood agar.

Eventually it was found that by adding the glucoside, æsculin, to the dye medium, the colonies of resistant organisms could be distinguished from those of streptococci.

Harrison and Vanderleck (1909) were able to recognise colonies of *Bact. coli* in æsculin-iron citrate agar by their black colour. This was due to the fact that the colon bacillus acted on æsculin with the production of glucose and æsculetin, which formed a black compound in the presence of iron. Diernhofer (1932) and S. J. Edwards (1932) also used æsculin for the differentiation of milk-souring streptococci (*Str. lactis*) from mastitis streptococci.

The dye-resistant organisms mentioned acted on æsculin in the presence of ferric citrate in the same way as *Bact. coli*, and

their colonies could be distinguished from those of mastitis streptococci by their black colour. The optimal amount of each of these two substances was found to be 0·05 gm. per cent. When higher amounts were used the blood agar medium became intensely discoloured and the development of streptococci was affected. More recently, even better results were obtained by adding æsculin alone to a concentration of 0·1 per cent. In this case the colour of the colonies was not so intense as when ferric citrate also was present, but there was practically no diffusion into the surrounding medium. After 48 hours' incubation at 37° C. the colonies of contaminating organisms were usually larger than those of mastitis streptococci and could be distinguished by their black colour, readily detectable by reflected light against a white background. (Fig. 2.) A few *Bact. coli* strains produced a less distinct colour but they could be recognised by the size of their colonies.



*a.* *b.*

FIG. 2.

(*a*) Blood agar with 1 : 500,000 crystal violet.
(*b*) The same with 0·1 per cent. æsculin.
The dye-resistant staphylococcus present in both media has produced black colonies in medium (*b*).

## COMPOSITION AND USE OF THE FINAL MEDIUM.

The medium finally recommended has the following composition :—

| | |
|---|---|
| Meat extract (Lemco) agar *p*H 7·4 ... ... ... | 1,000 c.c. |
| Crystal violet (B.D.H.) 0·1 per cent. ... ... | 2 c.c. |
| Defibrinated ox blood ... ... ... ... | 50 c.c. |
| Æsculin ... ... ... ... ... ... | 1·0 gm. |

When a large number of milk samples are to be examined, the present practice is to add the three last-named components to a flask containing one litre of the melted agar kept at a temperature

of 50° C. The æsculin should first be brought into solution by boiling in a small quantity of water. The final medium is drawn off under pressure from the flask, in approximately 10·0 c.c. quantities, into plates containing saline dilutions of the milk sediment. The plates are well shaken to ensure even mixing and incubated for 48 hours at 37° C., when they are read.

With this technique a large number of milk samples can be plated without much labour. If the samples have been taken under reasonably sterile precautions and the milks kept at a low temperature until they are sown into plates, it is a comparatively simple matter to detect those samples containing mastitis streptococci. The majority of the contaminating organisms present in milk are inhibited by the crystal violet, while the more common dye-resistant organisms can be distinguished by the dark appearance of their colonies. Group I mastitis streptococci, being often beta-hæmolytic, can be recognised by the presence of colonies surrounded by narrow zones of hæmolysis (Figs. 1 and 2). Non-hæmolytic streptococci belonging to Groups I and II appear as uniformly small colonies which produce no change in the surrounding medium. Such colonies ought to be identified by transferring to broth and their action tested on milk containing 1 :20,000 methylene blue, mannite and salicin.

## Summary.

By taking advantage of the fact that streptococci are more resistant to the bactericidal action of crystal violet than the majority of milk saprophytes, a selective medium has been devised which has afforded satisfactory results in the cultural diagnosis of bovine mastitis. Organisms which are not inhibited by the dye can be differentiated in blood agar from mastitis streptococci by the inclusion of æsculin, upon which they act to produce black colonies.

## References.

Bischoff, O. 1930. *Zbl. Bakt. I. (Orig.)*, **117**, 396.

Bryan, C. S. 1932. *Amer. J. Publ. Health*, **22**, 749.

Churchman, J. W. 1912. *J. Exp. Med.*, **16**, 221.

Diernhofer, K. 1932. *Zschr. Infekts. Haust.*, **42**, 67; *Ibid.*, 1932, *Milchwirts. Forsch.*, **13**, 368; *Ibid.*, 1933, *Zschr. Fleisch- u. Milchhyg.*, **43**, 225.

Edwards, S. J. 1930. *Proc. Roy. Soc. Med.*, **24**, 1369; *Ibid.*, 1932, *J. Comp. Path. & Therap.*, **45**, 43.

Harrison, F. C., and Vanderleck, J. 1909. *Zbl. Bakt. I. (Orig.)*, **22**, 547.

Haxthausen, H. 1927. *Ann. Derm. Syph.*, **8**, 201.

Klimmer, M., Haupt, H., and Roots, E. 1928. *Zbl. Bakt. I. (Orig.)*, **107**, 206.

Krumwiede, C., and Pratt, J. S. 1914. *J. Exp. Med.*, **19**, 20.

Minett, F. C., Stableforth, A. W., and Edwards, S. J. 1930. *J. Comp. Path. & Therap.*, **43**, 163.

Seelemann, M. 1928. *Archiv. f. Tierheilk.*, **58**, 1.

# THE OCCURRENCE OF *BABESIA MOTASI* WENYON, 1926, IN SHEEP IN NORTHERN NIGERIA, WITH A DISCUSSION ON THE CLASSIFICATION OF THE PIROPLASMS.

By

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The Discovery, Clinical Features and Distribution of *Babesia motasi* Wenyon, 1926=*Piroplasma ovis* s. str. (Lestoquard, 1925).

THIS piroplasm of the sheep was first described by Motas (1903) in Roumania, where it caused a serious disease known as "carceag" associated with hæmoglobinuria. The infection, however, may occur in two clinical types, namely, a severe form and a mild form. The serious type was characterised by fever, prostration, hæmoglobinuria, and profound anæmia. The red blood cells might fall from 8,000,000 to 1,500,000 per cubic millimetre. *Post-mortem* examination revealed glandular enlargement and a soft spleen double the normal size. There was congestion of the intestinal mucosa, especially marked in the cæcum.

Motas described the parasite as seen in the peripheral blood as follows: "Dans le sang de malades on troûve un nombre variable de globules rouges renfermant des piroplasmes identiques quant à leur morphologie et à leur biologie, à ceux de l'hemoglobinurie du bœuf (fièvre du Texas)." Dschunkowsky and Luhs (1909) figured this parasite in sheep in Transcaucasia and Lestoquard (1925) described it in Algeria. According to Donatien and Lestoquard (1930), it has also been found in Dalmatia. The parasites measure 2·5$\mu$ to 4$\mu$ in length by 1·2$\mu$ to 3$\mu$ in breadth.

The Occurrence of *Babesia motasi* (*Piroplasma ovis*) In Northern Nigeria.

In two sheep, both of which had been imported from French West Africa, parasites were found in the peripheral blood morphologically identical with *Babesia motasi*. Both animals were ill with symptoms that were not characteristic of any particular infection and the parasites were discovered accidentally in the usual routine blood examinations. This parasite has never been found in the routine examination of many hundreds of locally bred sheep, which, however, are commonly infected with *Babesia ovis* (*Babesiella ovis*). As far as can be ascertained, there seems

to be no reference in the literature to the occurrence of *Babesia motasi* (*Piroplasma ovis*) in either East or South Africa, and until now it has not been reported in West Africa. As this infection has only been found in two imported sheep in Northern Nigeria the question arises as to where the infection was acquired.

One of the sheep infected had been in Northern Nigeria for over a year and had been dipped regularly. In the first film examined from this animal only a few parasites were found but on the third day the parasites were numerous. Parasites disappeared from the peripheral blood but the animal died on the seventh day. The principal features noted at the *post-mortem* were a greatly enlarged, soft, and congested spleen and, further, there was marked congestion of the spleen and kidneys. There was no evidence of hæmoglobinuria.

Smears of the spleen showed that many of the mononuclears had ingested parasitised red cells (Fig. 6). Sections of the spleen showed the presence of parasites in large numbers, while in sections of the kidneys many of the capillaries were completely blocked with masses of parasitised red cells.

In the second sheep infected with *Babesia motasi* a few parasites were found in the peripheral blood on one occasion and the infection then apparently disappeared. This animal did not exhibit any symptoms other than a rise of temperature and loss of appetite for three days, after which the animal made a satisfactory recovery.

Inoculations of healthy, locally-bred sheep with blood containing *Babesia motasi* in large numbers failed to produce any evident infection or reaction.

It is of interest to note that *Babesia ovis* (*Babesiella ovis*) in the local breed of sheep in Northern Nigeria is a common infection, quite unassociated with any symptoms of sickness. In fact, these animals seem to possess a premunition towards this parasite.

From the above observations it would seem probable that the infection with *Babesia motasi* was imported, probably from the North, and the negative results obtained by the inoculation of locally-bred sheep points to a resistance of these to this parasite.

Morphology of *Babesia motasi* (*Piroplasma ovis*) as seen in Northern Nigeria.

The morphology of the parasite as seen in these two imported sheep in Northern Nigeria corresponded in every way to the description of *Babesia motasi* (*Piroplasma ovis*). Parasites appear singly or in pairs separated from one another, but frequently joined at their pointed ends, so forming an acute angle to one another (Figs. 1-5). On measurement they varied from 2·5$\mu$ to 3·5$\mu$ in length by 1·2$\mu$ to 1·5$\mu$ broad. The nucleus was large, rich in chromatin and frequently in two masses (Fig. 1).

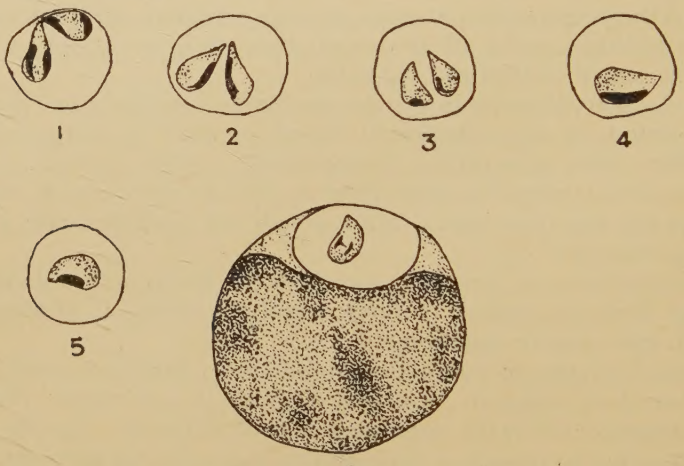
Phagocytosis was well marked in the spleen (Fig. 6). The

effect of the parasite on the red cells varied. Most of the infected cells were normal in shape and size while others were conspicuously enlarged. A few red cells were much distorted.

*A Discussion of the Classification of the Piroplasmata.*

The confusion which has arisen in the nomenclature of this group of important organisms owing to the diverse views of various workers renders it necessary to discuss the classification in some detail.

França (1917) (1918) classified the Piroplasmata and recognised a number of genera. The first careful systematic study was that of du Toit (1918), who modified França's scheme and retained two families, namely the Babesidæ, comprising the genera *Babesia, Piroplasma, Nicollia, Nuttallia, Smithia, Rossiella* and *Gonderia,* and the family Theileridæ, containing two genera, *Theileria* and *Rangelia.*




*B. Motasi.* ×3,000. W. Cooper, Pinx.

Figures drawn to a magnification of approximately 3,000 diameters.
Figs. 1-5.—*Babesia motasi* in the peripheral blood of a sheep.
Fig. 6.—Phagocytosis of *Babesia motasi* by a mononuclear cell of the spleen.

The classification of du Toit of the species in domestic animals is as follows :—

I. Family : Babesidæ Poche, 1913.

(1) Genus : *Babesia* Starcovici, 1893.

(*a*) Sub-genus : *Babesia* (Starcovici, 1893). Small forms of the parasite under 2·5$\mu$ in length. Multiplication by division into two.

Species : *B. bovis* (Babes, 1888) in cattle.
*B. ovis* (Babes, 1892) in sheep.
*B. argentina* (Lignières, 1909) in cattle.
*B. gibsoni* (Patton, 1910) in dogs and jackals.
*B. divergens* (M'Fadyean and Stockman, 1911) in cattle.

(*b*) Sub-genus: *Piroplasma* (Patton, 1895).
Large parasites over 3$\mu$ in length which contain two nuclear masses. Parasites are typically pear-shaped and divide into two by a budding process.
Species: *P. bigeminum* (Smith and Kilborne, 1893) in cattle.
*P. caballi* (Nuttall, 1910) in horses.
*P. trautmanni* (Knuth and du Toit) du Toit (1918) in pigs.

(2) Genus: *Nuttallia* França, 1910.
Parasites small oval or pear-shaped organisms which divide into four arranged into the form of a cross.
Species: *N. equi* (Laveran, 1901), horse.
N. sp? (Dschunkowsky and Luhs, 1909), sheep in Transcaucasia.
N. sp.? (Dschunkowsky and Luhs, 1913), horse in Transcaucasia.

(3) Genus: *Nicollia* Nuttall, 1908.
Small oval or pear-shaped parasites which divide into four daughter cells arranged in a four-leafed fashion in the red cells.
Species: *N. quadrigemina* (Nicolle, 1907) in the gondi.
(This genus is not described in domestic animals.)

(4) Genus: *Smithia* França, 1910.
Parasites are pear-shaped and stretch completely across the cell. Division into the form of a cross.
Species: *Smithia microti* (França, 1910) in field mouse.
*Smithia talpæ* (Galli-Valerio, 1913) in mole.
(This genus is not described in domestic animals.)

(5) Genus: *Rossiella* Nuttall, 1912.
Parasites are large and rounded in shape. One to four parasites occur in a red cell.
Species: *Rossiella rossi* (Nuttall, 1910), dog.

(6) Genus: *Gonderia* du Toit, 1918.
Parasites of small size. Division into four daughter cells comprised mostly of chromatin and with a tendency to form a cross. (Koch's blue bodies have not been found.)
Species: *G. mutans* (Theiler, 1906), cattle.
*G. hirci* (Dschunkowsky and Luhs, 1909), goat.
*G. buffeli* (Neveu-Lemaire, 1912), buffalo.
*G. camelensis* (Yakimoff and Schokhor, 1917), camels.

II. Family: Theileridæ, du Toit, 1918.

(1) Genus: *Theileria*.
The parasites are minute and rounded or rod-shaped organisms. Schizogony occurs in the cells of the lymphatic system (Koch's blue bodies).
Species: *T. parva* (Theiler, 1904), cattle.
*T. annulata* (Dschunkowsky, 1904), cattle.
*T. ovis* (Littlewood, 1914), sheep.

(2) Genus: *Rangelia* Carini and Maciel, 1914.
Parasites are round, oval or pear-shaped. Multiplication by

division into two occurs in the red cells or by schizogony in the endothelial cells.

Species: *R. vitalii* (Rangelia, Pestana, 1910), dogs in South America.

Wenyon (1926) critically reviewed and completely revised this classification. He placed these organisms in the sub-order Piroplasmiidea and, as suggested by du Toit (1918), retained two families, namely the Babesidæ and the Theileridæ. He considered there was not sufficient grounds for the retention of these various generic names, which were based mainly on differences of size, shape and division into two or four daughter cells. All the organisms in the family Babesidæ agree with one another in that they consist of cytoplasm and chromatin and produce no pigment.

Wenyon points out that if these early methods are adopted for the formation of different genera serious confusion will arise. For example, two important groups of organisms, namely the *Plasmodia* and the *Trypanosoma* might be further sub-divided into a number of genera if variations in morphology were considered. Wenyon rightly considers that such differences should not be used for sub-dividing a group into genera and that nothing can be gained by adopting this method in the present state of our knowledge, especially when it is realised that in no single instance is the complete life-cycle in the invertebrate known.

Wenyon (1926) rejects the generic names *Piroplasma, Nicollia, Nuttallia, Smithia, Rossiella, Gonderia,* and the generic names *Babesiella* of Mesnil (1919) and *Microbabesia* of Sohns (1918) and includes these in the single genus *Babesia*. In the family Theileridæ he retains the single genus *Theileria* and rejects the generic name *Rangelia canis* on the grounds that there is not sufficient evidence at present to consider this organism as different from *Babesia canis*. Wenyon's classification is as follows:—

Sub-order: Piroplasmiidea.

I. Family: Babesidæ Poche, 1913.
Genus: *Babesia* Starcovici, 1893.
Synonyms: *Piroplasma, Nicollia, Nuttallia, Smithia, Rossiella, Gonderia, Babesiella,* and *Microbabesia.*

II. Family: Theileridæ du Toit, 1918.
Genus: *Theileria* Bettencourt França and Borges, 1907.
Synonym: *Rangelia.*

This classification, which is based on essential morphological characteristics, is in the present state of our knowledge justified and reduces to simplicity the admittedly confused nomenclature. In our opinion this is the only rational method so far set forth in the classification of this group.

In adopting a single genus *Babesia* in the family Babesidæ Wenyon (1926) drew attention to the fact that both in cattle and sheep the parasites could be separated according to size into three

groups, namely large forms, intermediate forms, and very small forms. The species in cattle became *Babesia bigeminum* (largest forms) *Babesia bovis* (intermediate forms) and *Babesia mutans* (smallest forms). The species in sheep were named *Babesia motasi* Wenyon, 1926 (largest forms), *Babesia ovis* (intermediate forms), and *Babesia sergenti* Wenyon, 1926 (smallest forms).

More recent observations, however, on the smallest parasites in cattle and sheep indicate that these cannot be retained in the genus *Babesia*. It is necessary, as will be seen later, to remove *Babesia mutans* and *Babesia sergenti* into the family Theileridæ.

Donatien and Lestoquard (1930), two French authorities on piroplasmosis, have reviewed the piroplasms of domestic animals and disagree entirely with Wenyon's rejection of certain genera. They adopt two families, namely the Piroplasmidæ and the Theileridæ, and consider that on morphological, biological, epidemiological and pathological characteristics three genera should be retained for the piroplasms of sheep and cattle. Their classification is as follows:—

*Classification of Donatien and Lestoquard.*

I. Family: Piroplasmidæ.

(*a*) Genus: *Piroplasma*
- Sub-genus *Piroplasma*
  - *P. bigeminum* (cattle)
  - *P. ovis* (sheep)
  - *P. caballi* (horse)
  - *P. canis* (dog)
  - *P. trautmanni* (pig)
- Sub-genus *Babesiella*
  - *B. bovis* (cattle)
  - *B. argentina* (cattle)
  - *B. berbera* (cattle)
  - *B. major* (cattle)
  - *B. ovis* (sheep)

(*b*) Genus: *Nuttallia* ... ... ... *N. equi* (horse)

II. Family: Theileridæ
- *T. mutans* (cattle)
- *T. parva* (cattle)
- *T. dispar* (cattle)
- *T. ovis* (sheep) pathogenic
- *T. recondita* (sheep) non-pathogenic

In the above classification the French observers contend that size, morphology and position of the parasites in the red cells justify the retention of these genera. Biological differences may be well marked. Rapid passage of *Piroplasma ovis* increases the virulence, but this does not occur in the transmission of *Babesiella ovis*. Trypan blue is a specific in the treatment of *Piroplasma* infection but has absolutely no action on *Babesiella*. On this latter feature alone Donatien and Lestoquard state there is sufficient grounds for retaining a separate generic name.

In *Babesiella* characteristic pathological changes occur in the kidneys (large black kidneys) which are never seen in *Piroplasma*

infections. The distribution of the genus *Piroplasma* is widespread, occurring in widely separated continents and areas, whereas *Babesiella* is limited in its species distribution to a single continent or region. Lastly, *Piroplasma* occurs in all species of mammals, whereas *Babesiella* is confined exclusively to ruminants (ox, sheep and goat).

For the above reasons Donatien and Lestoquard conclude that the genus *Babesia* adopted by Wenyon is partially synonymous with the genera or sub-genera *Piroplasma, Babesiella, Gonderia* and *Nuttallia*. This difference in the generic and specific names adopted by Wenyon (1926) and Donatien and Lestoquard (1930) for these parasites in domestic animals (cattle, sheep, horses and dogs) may be illustrated as follows :—

| Wenyon (1926). | | Donatien and Lestoquard (1930). |
|---|---|---|
| (1) *Babesia motasi* (Large form of parasites in sheep) | = | *Piroplasma ovis* |
| (2) *Babesia ovis* (Intermediate form in sheep) | = | *Babesiella ovis* |
| (3) *Babesia sergenti* (Smallest form in sheep) | = | *Theileria recondita* |
| (4) *Theileria hirci* (Theileriasis in goats and sheep) | = | *Theileria ovis* |
| (5) *Babesia bigemina* (Large form in cattle) | = | *Piroplasma bigeminum* |
| (6) *Babesia bovis* (Intermediate form in cattle) | = | *Babesiella bovis*<br>,, *argentina*<br>,, *berbera*<br>,, *major* |
| (7) *Babesia mutans* (Smallest form in cattle) | = | *T. mutans.* |
| (8) *Babesia caballi* (horse) | = | *P. caballi* |
| (9) *Babesia equi* (horse) | = | *Nuttallia equi* |
| (10) *Babesia trautmanni* (pig) | = | *Piroplasma trautmanni.* |

More recently du Toit (1930) has given a complete historical review of the literature dealing with Theileriasis in cattle and sheep. This valuable contribution has done much to help workers to a clear understanding of this important group of organisms. The description of *Theileria* (*Piroplasma*) *parva* by Theiler (1904), of *Theileria* (*Piroplasma*) *annulata* by Dschunkowsky and Luhs in 1904, and of *Theileria* (*Piroplasma*) (*Gonderia*) *mutans* by Theiler in 1906, was followed by a study of similar parasites in many parts of the world. In 1924 Sergent and his collaborators described a fourth species in cattle *Theileria dispar* in Algeria. Du Toit (1930) discusses the question as to whether all cases of Theileriasis which have been described in the literature fit into the four types mentioned above, and comes to the conclusion that there are intermediate stages between these types. Altogether there are

at least eight types of Theileriasis in cattle which can be distinguished, and du Toit has arranged these in their order of virulence as follows :—

(1) *Theileria parva* occurs in South Africa with a mortality of 90 per cent. to 100 per cent. Koch's blue bodies constantly present.
(2) Theileriasis. East Africa. Virulence low with a mortality of about 15 per cent. in calves.
(3) *Theileria dispar.* Algeria. Mortality in cattle, 20 per cent., 50 per cent. to 90 per cent. An acute disease followed by disappearance of parasites from the blood after recovery.
(4) Theileriasis. Morocco. Infection is transmissible with blood. A chronic or mild infection capable of becoming acute if animals are subjected to adverse conditions.
(5) *T. annulata.* Tunis. (Brumpt). Mortality, 5 per cent., 10 per cent. to 20 per cent. A chronic infection with a persistence of parasites in the blood. Disease may become acute. Koch's blue bodies fairly frequent during acute attacks.
(6) Theileriasis (i) N.E. Rhodesia. (Koch's blue bodies found.)
(ii) India. (Koch's blue bodies found.)
(iii) Egypt. (No Koch's bodies found.)
A few deaths may occur in susceptible animals.
(7) *T. mutans.* South Africa and Algeria. Strains with Koch's blue bodies.
(8) *T. mutans.* South Africa and Algeria. Strains without Koch's blue bodies.

In (7) and (8) 999 cases out of 1,000 produce no symptoms or very slight anæmia. Under stress of circumstances Koch's blue bodies may appear in small numbers. It would appear that *T. mutans* in (7) and (8) are identical.

In the family Theileridæ there are therefore four named species in cattle, namely *T. parva*, *T. dispar*, *T. annulata* and *T. mutans*, which differ from one another in their virulence. It is considered by du Toit that the name *Gonderia mutans* should be replaced by the name *Theileria mutans*, and Wenyon's name *Babesia mutans* should also be replaced by *Theileria mutans*.

Doubt is expressed by du Toit (1930) whether the four species of *Theileria* of cattle are " good " species. They might all be regarded as varieties or strains of one species—*T. parva*, but it is suggested that at present a more conservative course be taken by provisionally accepting the four species already named. On purely morphological grounds it is impossible to distinguish these species.

In sheep and goats two species of *Theileria* may be accepted provisionally, one of which is non-pathogenic and the other pathogenic. Morphologically, however, these are indistinguishable. The non-pathogenic form, like *Theileria* in cattle, is associated with the presence of scanty Koch's blue bodies. Rodhain (1916) described a parasite in sheep which did not seem to produce Koch's blue bodies and thus resembled *T. mutans* in that it was

non-virulent. This parasite in sheep Rodhain considered as a new species and he named it *Theileria ovis*. Yakimoff (1916) commented on Rodhain's paper and stated that at the third veterinary conference for Russia at Kharkov in 1913 he had already suggested the name *Theileria ovis* for this parasite. In this statement Yakimoff (1916) gives no reference to a publication, and we have been unable to trace in the literature any reference to a published account by Yakimoff in which the specific name suggested by him was mentioned.

The name for the non-pathogenic *Theileria* in sheep and goats would seem, therefore, to be *Theileria ovis* Rodhain, 1916. Sergent, Parrot and Hilbert (1922) named this parasite *Gonderia ovis,* but Lestoquard (1929), having described Koch's blue bodies in such infections, renamed the parasite *Theileria recondita*. Wenyon (1926), in view of the fact that Koch's blue bodies had not been described in the non-pathogenic form, considered it justifiable to transfer this organism to the genus *Babesia* and in doing so suggested a new specific name *Babesia sergenti*.

According to the evidence available the correct name for the non-pathogenic *Theileria* of sheep is *Theileria ovis* Rodhain, 1916, and *Babesia sergenti* Wenyon, 1926 and *Theileria recondita* Lestoquard, 1929 become synonyms.

The pathogenic form in sheep, with Koch's blue bodies, was first described in Sudanese sheep by Mason, 1915, and the following year in Egyptian sheep by Mason, 1916. Mason placed this parasite in the genus *Theileria* but did not give a specific name. In 1918, however, du Toit in his classification of the piroplasms gives this parasite the name *Theileria ovis* and attributes the name to Littlewood, 1914. Donatien and Lestoquard (1930), without giving any references, again quote *Theileria ovis* Littlewood, 1914, as the correct name for the pathogenic form in sheep and goats. The only justification for giving the name of Littlewood seems to be the fact that in his annual report he incorporated the work of Mason. This report was actually published in 1915. As far as can be ascertained there is no mention of species in Mason's account of this parasite either in 1915 or 1916, so that it would seem that neither Littlewood nor Mason named this species.

On the other hand, du Toit (1918) records the name *Theileria ovis,* which he attributes to Littlewood (1914). As the specific name *ovis* had, however, been already used for the non-pathogenic form *Theileria ovis* by Rodhain in 1916, it would seem that the correct name for the pathogenic form in sheep and goats is *Theileria hirci* Dschunkowsky and Urodschevich, 1924, as correctly pointed out by Wenyon (1926). The name *Theileria ovis* wrongly attributed to Littlewood in 1914 by du Toit must be abandoned.

## Conclusions Regarding the Classification.

In the opinion of the writers it seems essential and a matter of extreme importance that some general agreement amongst workers in these diseases in animals should be concluded. The urgency of this must be obvious to veterinarians and zoologists all over the world. It is the sub-division into genera that has led to the present confusion in the nomenclature. The question of species is at present a matter of less importance. We are in complete agreement with Wenyon (1926) that in the family Babesidæ the single generic name *Babesia* should, in the present state of our knowledge, be retained for the piroplasms of domestic animals. The names *Piroplasma*, *Babesiella* and *Nuttallia* should be considered as synonyms of *Babesia*.

We are not in agreement with Donatien and Lestoquard (1930) that these organisms should be subdivided into genera on the grounds brought forward by them. If such methods as reaction to drugs, etc., were adopted generally for the distinction of genera, it would obviously become necessary to reconsider the subdivision of the genus *Trypanosoma* and other protozoa.

Again, in the present state of our knowledge it would seem that there is general agreement that there is only a single genus in the family Theileridæ, namely *Theileria*.

The various morphological differences in the family Babesidæ, such as size, shape and division, justify the differentiation into two species in cattle, namely *Babesia bigemina* and *Babesia bovis*. On morphology alone it is impossible to differentiate *Babesia argentina*, *Babesia berbera*, and *Babesia major* from *Babesia bovis*. It is quite possible that these species are simply strains of *Babesia bovis* which vary in virulence. As suggested by du Toit (1930), we may retain provisionally the four species of *Theileria* in cattle. It is a matter of doubt, however, whether *Babesia argentina*, *Babesia berbera*, *Babesia major*, *Theileria dispar*, *Theileria annulata*, and *Theileria mutans* are " good " species.

We suggest the following classification for the piroplasmata of sheep and goats based on the adoption of the generic names *Babesia* and *Theileria*.

*Babesia argentina* was given the specific name in 1909 by Lignières and was not named in 1903 as has been erroneously stated. The organism was described by Lignières in 1903 but was not specifically named until 1909.

Sub-order : Piroplasmidæ.

Family : Babesidæ Poche, 1913.

Genus : *Babesia* Starcovici, 1893. Synonyms : *Piroplasma*, *Babesiella*, *Microbabesia*, *Nuttallia*, *Nicollia*, *Smithia*, *Rossiella*, *Gonderia* and *Françaiella*.

Species of *Babesia* in Cattle:

- *Babesia bigemina* (Smith and Kilbourne, 1893). Cattle.
  - Synonym: (*Piroplasma bigeminum*).
- *Babesia bovis* Starcovici, 1893. Cattle.
  - Synonyms: *Piroplasma divergens* (M'Fadyean and Stockman, 1911), *Babesiella bovis*.
- *Babesia argentina* Lignières, 1909, in cattle.
- *Babesia berbera* Sergent, Donatien, Parrot, Lestoquard, Plantureux, Rougebief, 1924.
  - Synonym: *Françaiella caucasica*.
- *Babesia major* Sergent, Donatien, Parrot, Lestoquard and Plantureux, 1924.
  - Synonym: *B. colchica*.

Species of *Babesia* in Sheep:

- *Babesia motasi* Wenyon, 1926.
  - Synonym: *Piroplasma ovis* (Lestoquard, 1925).
- *Babesia ovis* Starcovici, 1893.
  - Synonyms: *Babesiella ovis*, *Piroplasma hirci* Dschunkowsky and Luhs, 1909.

Family: Theileridæ du Toit, 1918.

Genus: *Theileria* Bettencourt, França and Borges, 1907.

Species of *Theileria* in Cattle:

- *T. parva* (Theiler, 1904).
- *T. dispar* Sergent and collab., 1924.
- *T. annulata* (Dschunkowsky and Luhs, 1904).
  - Synonym: *T. turkestanica* Oboldueff and Galouzo, 1928.
- *T. mutans* (Theiler, 1906).
  - Synonyms: *Babesia mutans* Wenyon, 1926, *Gonderia mutans* du Toit, 1918.

Species of *Theileria* in Sheep:

- *Theileria hirci* Dschunkowsky and Urodschevich, 1924.
  - Synonym: *Theileria ovis* du Toit, 1918.
- *Theileria ovis* Rodhain, 1916.
  - Synonyms: *Babesia sergenti* Wenyon, 1926; *Gonderia ovis* Lestoquard, 1924; *Theileria recondita* Lestoquard, 1929.

## References.

Babes, V. 1888. Sur l'hémoglobinurie bactérienne du boef. C.R. Acad. Sci., cvii, 692.

Babes, V. 1892. L'étiologie d'une enzootic des moutons denommée "Carceag" en Roumanie. C.R. Acad. Sci., cvx, 359.

Bettencourt, A., França, C., et Borges, J. 1907. Un cas de Piroplasmose bacilliforme chez le daim. Arq. R. Inst. Bact., Camara Pestana, i, 341.

Carini, A., et Maciel, J. 1914. Sobre a molestia dos câes, chamada Nambi-Uvu e o seu parasita (Rangelia vitalii). Ann. Paulistas de Med. e. Cirugia, iii, 65.

Donatien, A., et Lestoquard, F. 1930. De la classification des Piroplasmes des Animaux domestiques. Recueil de Méd. Vétér. Exotique, iii, 5.

Dschunkowsky, E., et Luhs, J. 1904. Die Piroplasmosen der Rinder. (Vorl. Mitt). Centralbl. Bakt. I. Abt., xxxv, 486.

Dschunkowsky, E., et Luhs, J. 1909. Protozoenkrankheiten des Blutes der Haustiere in Transkaukasien. 9° Congr. Internat. Méd. Vét. La Haye. p. 31, plate i, figs. 1 to 15.

Dschunkowsky, E., et Luhs, J. 1913. *Nuttallia* und *Piroplasma* bei der Piroplasmose der Einhufer in Transkaukasien. Parasitology, v, 289.

Dschunkowsky, E., et Urodschevich, V. 1924. Theileriasis in goats, sheep, and cattle, with a description of *Theileria hirci,* n. sp. from Serbia. Parasitology, xvi, 107.

Du Toit, P. J. 1918. Zur Systematik der Piroplasmen. Arch. f. Protistenkunde, xxxix, 84.

Du Toit, P. J. 1930. Theileriasis. Report Sect. Meetings Eleventh International Veterinary Congress, iii, 539.

França, C. 1910. Sur la classification des piroplasms et description de deux forms de ces parasites. Arq. R. Inst. Bact., Camara Pestana, ii, 89.

França, C. 1917. Sur la classification des hémosporidies. Jour. Sci. Math. Fis. e. Nat. Accad. Sci. Lisbon, i, I.

França, C. 1918. Quelques considérations sur la classification des hématozoaires. Jour. Sci. Math. Fis. e. Nat. Acad. Sci. Lisbon, i, 221.

Galli-Valerio, B. 1914. *Smithia talpæ* n. sp. (Piroplasmidæ) chez *Talpa europæa.* L. Cbl. f. Bakt. I. Orig., Bd. 73, 8, 142.

Hall, G. N. 1931. Babesia of Sheep. Annual Report of the Veterinary Pathologist, Northern Nigeria.

Laveran, A., and Nicolle, M. 1899. Contribution à l'étude de *Pyrosoma bigeminum.* C.R. Soc. Biol., li, 800.

Laveran, A., and Nicolle, M. 1899. Hématozoaires endoglobulaires du mouton. C.R. Soc. Biol., li, 800.

Laveran, A. 1901. Contribution to the study of *Piroplasma equi.* C.R. Soc. Biol., liii, 385.

Lestoquard, F. 1924. Les piroplasmoses du mouten en Algérie. (Note préliminaire.) Bull. Soc. Path. Exot., xvii, 122.

Lestoquard, F. 1924. Deuxième note sur les piroplasmoses du mouton en Algérie. L'anaplasmose, *Anaplasma ovis* nov. sp. Bull. Soc. Path. Exot., xvii, 784.

Lestoquard, F. 1925. Troisième note sur les piroplasmoses du mouton en Algérie. La piroplasmose vraie; *Piroplasma* (s. str.) *ovis* (nov. sp.). Comparaison avec *Babesiella ovis.* Bull. Soc. Path. Exot., xviii, 140.

Lestoquard, F. 1926. Les piroplasmoses du mouton et de la chèvre. Arch. Inst. Pasteur d'Algérie, iv, 222.

Lestoquard, F. 1929. Existence de " corps en grenade " dans le cycle évolutif de *Gonderia ovis.* C.R. Soc. Biol., c, 14, 1177.

Lignières, J. 1901. Sur la " Tristeza." Ann. Inst. Past., xv, 121.

Lignières, J. 1903. La Piroplasmose bovine. Nouvelles recherches et observations sur la multiplicité des parasites, leur évolution, la transmission naturelle de la maladie et la vaccination. Arch. de Parasit., 7, 398.

Lignières, M. J. 1909. La prophylaxe et la pathologie des maladies protozoaires (piroplasmoses, trypanosomes, etc.) avec demonstra-

tion des parasites spécifiques des animaux transmetteurs (tiques, moustiques, etc.). Neuvième Congrès international de medicine vétérinaire à la Haye, Sept., 1909.

Littlewood, W. 1915. Annual Report Veterinary Services, 1914, Govt. Press, Cairo.

Mason, F. E. 1915. Annual Report for the Year 1914, Veterinary Service, Ministry of Agriculture. Veterinary Pathological Report, page 24. Government Press, Cairo.

Mason, F. E. 1916. Annual Report for the Year 1915, Ministry of Agriculture, page 41. Government Press, Cairo.

M'Fadyean, J., and Stockman, S. 1911. A new species of Piroplasm found in the blood of British cattle. Jl. Comp. Path. & Therap., xxiv, 340.

Mesnil, F. 1919. [On paper by Du Toit, P. J. 1918.] Bull. Inst. Past., xvii, 193.

Motas, C. 1903. La Piroplasmose ovine "carceag." C.R. Soc. Biol., liv, 1522.

Neveu-Lemaire. 1912. Parasitologie des animaux domestiques. Paris.

Nicolle, C. 1907. Sur une piroplasmose nouvelle d'un rongeur. C.R. Soc. Biol., lxiii, 213.

Nuttall, 1908. Piroplasmosis. The Harben Lectures. III. Jl. R. Inst. Pub. Health, xvi, 513.

Nuttall. 1910. On Hæmatozoa occurring in wild animals in Africa, etc. Parasitology, iii, 108.

Nuttall. 1912. Note on *Rossiella rossi* (Nuttall 1910) occurring in the jackal in British East Africa. Parasitology, v, 61.

Oboldueff, G., and Galouzo, J. 1928. La theiléiriose des bovides en Asie centrale. Ann. de l'Inst. Pasteur, xlii, 1470.

Patton, W. H. 1895. The name of the Southern or splenic cattle-fever parasite. Amer. Natural, xxix, 498.

Patton, W. H. 1910. Preliminary report on a new piroplasm (*Piroplasma gibsoni* sp. nov.) found in the blood of hounds of the Madras hunt and subsequently discovered in the blood of the jackal, *Canis aureus*. Bull. Soc. Path. Exot., iii, 274.

Pestana, B., and Rangel, R. 1910. *O. nambyuvu*. Rev. med. de San Paulo, xxii, 423.

Poche, F. 1913. Das System der Protozoa. Arch. f. Protist., xxx, 125.

Ratz, s. von. 1912. Über die Piroplasmose der Schafe. Allatorvosi Lapok, xxxv, 207.

Rodhain, J. 1916. Note sur les trypanoses et les piroplasmoses des grands animaux de l'Ouellé. Bull. Soc. Path. Exot., ix, 95.

Sergent, E., Parrot, L., and Hilbert, D. N. 1922. Morphologie de piroplasmes observés en Algérie chez le mouton (*Gonderia ovis*). Bull. Soc. Path. Exot., xv, 789.

Sergent, Ed., Donatien, A., Parrot, L., Lestoquard, F., Plantureux, E., and Rougebief, H. 1924. Etudes expérimentales sur les piroplasmoses bovines d'Algérie. Ann. Inst. Pasteur, xxxviii, 273.

Smith, T., and Kilborne, F. E. 1893. Investigations into the nature, causation, and prevention of Texas or Southern cattle fever. U.S. Dept. Agric. Bur. Anim. Indust. Bull., i, 177.

Sohns, J. C. F. 1918. *Microbusia divergens* in Nederlandsch-Indië. Nederl. Ind. Bl. V. Diergeneesk e. Dierent, xxx, 385.

Starcovici, C. 1893. Bemerkungen über den durch Babes entdeckten Blutparasiten und die durch denselben hervorgebrachten Krankheiten, die seuchenhafte Hämoglobinurie des de Rindes (Babes), das Texasfieber (Th. Smith) und der Carceag der Schafe (Babes). Centralbl. Bakt. I. Abt. xiv, 1.

Theiler, A. 1904. Rhodesian Tick Fever. Transvaal Agric. Journal, ii, 421.

Theiler, A. 1906. *Piroplasma mutans* (nov. sp.) of South African cattle. Jl. Comp. Path. & Therap., xix, 292.

Yakimoff, W. L. 1916. A propos de la Note de M. Rodhain sur *Theileria ovis*. Bull. Soc. Path. Exot., ix, 201.

Yakimoff, W. L., et Schokhor, N. J. 1917. Theilériose des bovides. Bull. Soc. Path. Exot., x, 307.

Yakimoff, W. L. 1931. Uber Theileriosen. Report Sec. Meeting Eleventh International Veterinary Congress, iii, 574.

Wenyon, C. M. 1926. Protozoology. Baillière, Tindall and Cox, London.

# PATHOGENESIS OF BRUCELLOSIS BANG.

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## The Route of Infection.

A. *Clinical Data.*

In man infection with *Brucella* Bang is characterised by general sickness of a septic nature, usually with long persisting fever, enlargement of spleen and liver, neuralgiæ, and sometimes localisation in the genitals. Persons coming into contact with the virus repeatedly may show a typical cutaneous affection as an expression of acquired allergy.

In ruminants no dominating general symptoms appear; probably they do not even belong to the clinical picture. The disease is localised in the genitals (uterus, testis, epididymis); the outstanding symptom is abortion. The udder often remains a source of infection for a long period.

Horses may pass through a period of general sickness with high temperature, usually followed by pathological foci in ligamentous and tendinous tissue in definite areas with a tendency to suppuration.

As a rule infected dogs do not show clinical symptoms. In exceptional cases the genitals are infected (abscess in the epididymis, abortion).

Summarising, one may say that distinguishing reactions are provoked in man and animals by *Brucella* infection, *viz.*, especially general sickness in man, gynæcological symptoms in cows, surgical affections in horses, and as a rule no symptoms in dogs.

In order to study the pathogenesis of *Brucella*, it is important to determine where the virus is available in the organism.

In man *Brucella* Bang usually circulates in the blood for a considerable time, sometimes even for several months. Furthermore, it is found in the spleen and can be cultivated from the pus of pustules and furuncules and from the milk (own observation). Some investigators have succeeded in cultivating it also from the urine,[1] fæces and bile.[2]

In infected cows the abortion bacillus is lodged in the uterine wall and in the udder (sometimes many years *post-infectionem*). The micro-organism is also found in the so-called traumatic bursitis and in joint fluid;[3] in exceptional cases also in the urine, fæces, blood,[4] spleen and thyroid gland.[5]

In horses *Brucella* is often found in the serous contents of bursitis and tendo-vaginitis, sometimes in joint liquid, and regularly in the pus of boils about the neck and withers.[6]

In a dog exhibiting strong serum reactions against *Brucella* the bovine abortion bacillus was cultivated from the blood,[7] and in another one from an abscess of the epididymis.[8]

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B. *Experimental Infection.*

The following experiments have been carried out with the bovine type (Bang). As test animals dogs, guinea-pigs, a horse, and a goat were used.

I. *Horse.*

From the blood of a horse infected with *Brucella* Bang culture given with fodder, this micro-organism was cultivated three days after the first feed and for the second time three days after the fourth one, which was given 56 days after the commencement of the experiment. Between the thirteenth and seventy-fifth day after the first contaminated feed *Brucella* could not be found on 16 attempted blood cultures. This animal showed no clinical symptoms with the exception of a gradual rise of temperature during the first fortnight of the experiment. Local symptoms did not appear, not even after an attempt to provoke diminished tissue resistance by applying permanent pressure in the region of the withers.

Both in horses with the acquired disease and in those with spontaneous *Brucella* infection cultures were obtainable from the pus which was produced on the site of a subcutaneous injection of a dead *Brucella* suspension. This remarkable experience may perhaps be explained by supposing that the injection of micro-organisms caused an allergic focal reaction, from which bacteria escaped into the blood circulation, while localisation of the infection appeared at the site of injection (local reaction), where favourable conditions are afforded for a deposit of micro-organisms (decreased tissue resistance).

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II. *Goat.*

A full-grown he-goat infected in the conjunctival sac with 10 drops of a *Brucella* culture (first subculture from a guinea-pig) showed no agglutinins or complement-fixing bodies in the serum at the outset of the experiment, but the serum reacted vigorously with *Brucella* after seven days (agglutination titre 1,600, complement-fixation index 10).

This infection experiment was carried out with the intention to study the appearance of *Brucella* in the blood after the test animal had been infected. For this purpose 10 c.c. of blood were drawn at various intervals from the jugular vein into a flask, corked with a rubber stopper and containing 150 c.c. glucose-broth medium. This was kept in the incubator at 37° C. for six and 14 days respectively, and then inoculated on solid mediums,

which were brought into a bell-jar containing about 10 per cent. carbonic acid gas. The colonies were identified by their typical aspect (dome-shaped and nacreous lustre), originally bad or no growth at all in uncorked tubes, gram-negative staining, lack of mobility, and agglutination with specific serum.

TABLE I.

| *Hours after infection.* | | | | *Days after infection.* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1½ | 6½ | 23 | 30 | 2 | 3 | 4 | 5 | 7 | 11 | 12 | 14 | 35 |
| — | — | — | — | — | + | + | — | — | + | — | — | — |

*Brucella* was cultivated from the circulating blood three, four and eleven days respectively *post-infectionem*.

This animal was killed by chloroform narcosis 35 days after infection. One single colony of *Brucella* was cultivated from the mandibular lymphatic glands and numerous colonies from the spleen, whereas no positive cultures were obtained from the retropharyngeal and cranial cervical glands, kidney, and bone marrow.

At autopsy the liver was practically destroyed by *distomum* (cultures from the liver were intensely contaminated).

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III. *Dogs.*

Dogs can easily be infected either *per os* or in the conjunctival sac or intracutaneously.[7]

For bacteriological examination of the organs, the dogs were killed by means of chloroform-narcosis and immediately thereafter the *post-mortems* were performed with aseptic precautions. First of all the thoracic cavity was opened and blood obtained from the heart with a sterile syringe. The organs were removed from the cadaver with separate disinfected instruments and material from them was spread out on the culture medium (glass dishes containing 1 per cent. glycerin—1 per cent. glucose-broth-agar), which remained in a bell-jar containing about 10 per cent. carbonic acid gas at 37° C. for a period of four to five days. The bone-marrow was obtained from the femur. In several cases also guinea-pigs were infected subcutaneously with minced organs of these dogs. Five to 8 c.c. of blood were mixed with 100 c.c. solution of 1 per cent. glucose broth. The flasks were closed with rubber stoppers and kept in the incubator and the contents were used to inoculate solid mediums after eight days. When the cultures on agar were negative new ones were made after 16 days.

The following localisations were registered in the test dogs.

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| From | 23 | dogs | *Brucella* | was | cultivated | from | the | spleen ... ... ... | 15 | times |
| „ | 23 | „ | „ | „ | „ | „ | | liver ... ... ... | 12 | „ |
| „ | 22 | „ | „ | „ | „ | „ | | kidney ... ... | 1 | time |
| „ | 21 | „ | „ | „ | „ | „ | | bone-marrow ... ... | 10 | times |
| „ | 16 | „ | „ | „ | „ | „ | | mesenteric lymph glands | 8 | „ |
| „ | 7 | „ | „ | „ | „ | „ | | testis ... ... ... | 0 | time |
| „ | 18 | „ | „ | „ | „ | „ | | blood ... ... ... | 4 | times |
| „ | 7 | „ | „ | „ | „ | „ | | bile ... ... ... | 0 | time |

It is remarkable that the kidneys and testicles were very seldom infected, whereas the spleen, liver and bone-marrow, integral parts of the reticulo-endothelial system, were frequently involved. Only from one of the 21 cases were a few *Brucella* colonies cultivated from the kidney, while an abundant culture was obtained from the blood. In this case it is possible that the cultivation of the organisms from the kidney was not due to kidney infection, and that the bacilli were derived from the blood of the organ. It is therefore evident that one cannot accept localisation in the tissues of organs without reserve.

Out of seven specimens of bile examined *Brucella* could not be cultivated once. Two of these specimens were obtained from gall-bladders of livers which gave positive cultures.

The question arose whether the absence of *Brucella* in the kidney contrary to its presence in other organs must be ascribed to higher resistance of the kidney. This problem was attacked by infecting two puppies orally with the same amount of *Brucella* suspension, one of them having also been injected with 10 mg. uranyl nitrate two days previously, which provoked parenchymatous degeneration of the kidney (the urine contained many leucocytes, granular casts and some tubulo-epithelial cells and the reaction on albumen was weak positive; histological examination of the kidneys showed cloudy swelling of the tubular epithelium, parenchymatous degeneration). Eighteen days afterwards *Brucella* was cultivated from the kidney of the last-mentioned puppy and not from that of the other one. As the blood cultures were negative in the case of the uranyl nitrate poisoning, the deposit of micro-organisms in this organ was probably the result of damage of the kidney with consequent decreased resistance.

In another experiment a dog was infected *per os* with a culture of *Brucella*, and in order to provoke localisation it was simultaneously injected with a solution of silver nitrate into one of the testicles, which became necrotic. After a period of three weeks, however, *Brucella* was not found in this organ.

A puppy was infected in the conjunctival sac and at the same time a solution of silver nitrate was injected in one of the testicles.

Twenty-two days afterwards *Brucella* was cultivated from the spleen, mesenteric glands and bone-marrow, but not from the testicles (not even from the injected one, which had become necrotic).

None of the infected dogs in which *Brucella* was cultivated from the blood and several organs reacted with clinical symptoms to the infection. The temperature remained practically normal. Positive cultures in very large quantities were obtained from some of these cases, sometimes also in response to infection with a small amount of culture (five million bacteria *per os* or in the conjunctival sac).

(*a*) *Oral Infection.*

Twenty-four hours after oral infection with *Brucella*, it was cultivated in small quantities from the retropharyngeal lymphatic glands, but not from the submandibular, parotic, mesenteric, inguinal, axillary or tracheo-bronchial glands. At this stage multiple *Brucella* colonies could also be obtained, not only from the spleen but also from the blood. One puppy, killed three days after infection, had *Brucella* in large quantities in the spleen, less in the mesenteric glands, liver and tracheo-bronchial lymphatic glands (bifurcation glands), but none in the blood and " mandibulo-pharyngeal " ones* and these of the neck. Strange to say, no *Brucella* was found in blood and organs of two puppies six and ten days respectively *post-infectionem*. However, it was regularly cultivated after the fourteenth day from the spleen, liver, mesenteric glands and bone-marrow, and occasionally from the blood (on the eighteenth, twenty-fourth and thirty-second day). Seventy-four days *post-infectionem* the spleen still contained *Brucella* (*see* Table II).

As a rule the greatest number of colonies were obtained from the liver and spleen and to a smaller extent from the bone-marrow.

(*b*) *Percutaneous Infection.*

In five dogs a culture of *Brucella* was rubbed into the slightly eroded skin, with subsequent formation of agglutinins and complement-fixing bodies in three cases. The blood cultures, made in two of these cases after a period of eleven and twelve days respectively, were negative. Three days *post-infectionem* one of these cases, a puppy of six weeks, had *Brucella* in the spleen, liver and bone-marrow, but not in the blood and kidney.

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* By " mandibulo-pharyngeal " lymphatic glands must be understood the following group : 1 gl. retropharyngealis medialis et lateralis, 1 gl. mandibularis and 1 gl. parotidea.

TABLE II.

| *Days after infection.* | 1 | 3 | 6 | 10 | 14 | 18 | 18 | 18 | 23 | 24* | 32 | 51 | 63 | 74 | 84 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Spleen ... ... | ± | + | — | — | +‡ | + + | — | + | | + | + | + + | | +‡ | — |
| Liver ... ... ... | — | + 4 | — | — | + | + | + + | + | | + | + | + | — | — | — |
| Kidney ... ... | — | — | — | — | — | — | — | | | + 2 | — | — | — | — | — |
| Suprarenals ... ... | — | — | | | | | | | | | | | | | |
| Mesenteric glands ... | — | + 2 | — | — | +‡ | + | — | + | | + | + + | — | | | |
| Bone-marrow... ... | — | — | | — | +‡ | + | + | + | | + | + | — | | — | |
| Blood ... ... ... | + | + | + | + | — | — | + † | — | | | + | | | + | |
| " Mand.-pharyng." gl. | + 1 | + | | | | | | | | | | | | | |
| Inguinal glands ... | — | + | | | | | | | | | | | | | |
| Axillary glands ... | + | | | | | | | | | | | | | | |
| Bifurcation glands ... | — | + 3 | | | | | | | | | | — | | | |
| Testis ... ... ... | | | | | | — | | | | | | | | | |
| Agglutination titre ... | 0 | 0 | 800 | 200 | 1,600 | 3,200 | 3,200 | 800 | 400 | 800 | 1,600 | 1,600 | 400 | 1,600 | 200 |
| Compl.-fixation index§ | 0 | 0 | 6 | 9 | 9 | 10 | 10 | 10 | 7 | 10 | 10 | 10 | 8 | 10 | |
| Age at autopsy ... | 7w. | 7w. | 9w. | 9½w. | 10w. | 11w. | 11w. | 11w. | full grown | 9w. | 12w. | 15w. | 16w. | 18w. | 19w. |
| No. ... ... ... | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |

* 3 days previously 2nd oral infection.
† on the 8th day negative, but positive on the 16th day.
‡ only positive by inoculating guinea-pigs.
§ The complement fixation indices (1 to 10) correspond with positive reactions obtained with 0·06, 0·04, 0·02, 0·015, 0·01, 0·005, 0·003, 0·002, 0·001 and 0·0005 c.c. amounts of serum respectively.
In cases from which less than 10 colonies were cultivated, the amount is given behind the plus sign.

(*c*) *Conjunctival Infection.*

TABLE III.

| | *Days post-infectionem.* | | | | |
|---|---|---|---|---|---|
| | 10* | 11† | 22* | 60 | 135 |
| Spleen ... ... ... | — | +‡ | ++ | — | — |
| Liver ... ... ... | — | +‡ | — | — | — |
| Kidney ... ... ... | — | — | — | — | — |
| Mesenteric glands ... | — | — | + | | — |
| Bone-marrow ... ... | — | +‡ | + | — | — |
| Blood ... ... ... | — | | — | | |
| Testis ... ... ... | | | — | — | |
| Agglutination titre ... | 200 | 400 | 800 | 800 | 200 |
| Compl.-fixation index ... | 9 | 10 | 9 | 6 | 6 |
| Age at autopsy ... ... | 10w. | 7w. | 11w. | 14w. | 25w. |
| No. (continued) ... ... | 16 | 17 | 18 | 19 | 20 |

* 5 million bacteria in one conjunctival sac.
† in both conjunctival sacs 50 million bacteria, repeated 2 days afterwards.
‡ Only positive by inoculating guinea-pigs.

(*d*) *Contact Infection.*

Three puppies were infected by bringing them into contact with two orally infected ones.

TABLE IV.

| | *Number of days after having entered into infectious environment.* | | |
|---|---|---|---|
| | 42 | 61 | 105 |
| Spleen ... ... ... ... | + | ++ | ++ |
| Liver ... ... ... ... ... | $+_1$ | — | $+_2$ |
| Kidney ... ... ... ... | — | — | — |
| Mesenteric glands ... ... ... | | | + |
| Bone-marrow... ... ... ... | +* | — | — |
| Agglutination titre ... ... ... | 200 | 800 | 400 |
| Compl.-fixation index ... ... | 6 | | 9 |
| Age at autopsy ... ... ... | 13w. | 16w. | 22w. |
| Nr. (continued) ... ... ... | 21 | 22 | 23 |

* Only positive by inoculating guinea-pigs.

Opportunity for infection was therefore amply afforded to them, if there is any question of excreting micro-organisms at all. Indeed, excretion was found once in the urine of a dog, orally infected and killed after a period of 24 days (*see* Table II, No. 10).

No infection occurred in a healthy puppy brought together in a thoroughly disinfected cage with a dog orally infected on the previous day.

IV. *Guinea-pigs.*

Guinea-pigs had been infected subcutaneously for diagnostic purposes with material containing *Brucella* (milk, amniotic fluid, bursal and joint fluid of cows; pus, bursal and joint fluid of horses; organs of caviæ and dogs). At autopsy, six to eight weeks later, the micro-organisms were found in the following organs.

TABLE V.

| | *Number of animals used.* | *Positive cultures.* | *Percentage of positive cultures.* |
|---|---|---|---|
| In the spleen ... ... | 92 | 85 | 92 |
| In the liver ... ... | 61 | 31 | 50·8 |
| In the kidney ... ... | 39 | 11 | 28·2 |
| In the lymphatic glands ... | 30 | 23 | 76·7 |

The following investigations were performed in order to obtain more accurate data about the distribution of *Brucella* in the organism and the time at which infection of the organs occurs.

1. *Oral Infection.*

Full-grown guinea-pigs were infected orally with eight to ten drops of a *Brucella* suspension by means of a pipette. This suspension was obtained by washing off cultures of 48 hours growth on broth-agar (first subculture from the organs of dogs and guinea-pigs, in all about 100 to 5,000 million micro-organisms per c.c.). The guinea-pigs were killed by chloroform narcosis. The bacteriological examination of blood and organs was carried out as in the case of dogs.

(In several cases the investigation encountered difficulty due to stable infection with *Bact. bronchisepticus,* which were present in the bifurcation glands of apparently healthy guinea-pigs. Although this micro-organism resembles *Brucella* superficially, it can easily be distinguished from *Brucella* by its abundant growth under atmospheric conditions, appearance of concentric rings in older colonies, its mobility, and agglutination test.)

Very soon after the infection (one hour) the micro-organisms could already be traced in the " mandibulo-pharyngeal " glands, where they remained for at least three weeks and multiplied. Table VI gives a survey of its distribution in the lymphatic glands of this area. The micro-organisms were cultivated only once from the bifurcation glands, which was one hour after the infection (it is possible that a portion of the virus, though carefully administered, got into the trachea).

*Brucella* was found in the inguinal and axillary lymphatic glands in one case in a guinea-pig which was infected 22 days previously and whose blood also contained the bacilli.

TABLE VI.

| | *Oral Infection.* | | | | | | | | *Conjunctival Infection.* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Autopsy after* | *1 hour.* | *5 hours.* | *1½ days.* | *6 days.* | *9 days.* | *10 days.* | *15 days.* | *22 days.* | *1 day.* | *2 days.* | *4 days.* | *8 days* |
| 1. L. gl. retropharyngealis lateralis | +2 | — | +1 | + | — | + | ++ | +++ | + | — | + | +++ |
| 2. L. gl. retropharyngealis medialis | | +2 | | | — | | | + | — | — | | +5 |
| 3. L. gl. mandibularis ... ... | + | — | — | ++ | — | +++ | — | ++ | — | — | — | |
| 4. L. gl. parotidea ... ... | — | — | — | +++ | — | +1 | — | ++ | — | — | — | +2 |
| 5. L. gl. cervicalis cranialis ... | — | — | — | ++ | | — | — | | — | — | — | — |

In cases from which less than 10 colonies were cultivated, the amount is given behind the plus sign.

The micro-organisms appeared in the mesenteric glands and simultaneously in the blood nine days after infection.

The spleen and liver gave positive cultures two and three days respectively after infection, and afterwards *Brucella* was cultivated more or less regularly from these organs. In an exceptional case, in which *Brucella* appeared in the kidney in small quantities five days after infection, positive cultures could only be obtained from this organ when the blood culture was positive. The blood of the first-mentioned animal, which died spontaneously from pneumonia, was not tested, so that in this case also circulation of the micro-organisms in the blood may have occurred.

From the suprarenals a culture of *Brucella* was obtained only in two cases out of the 27 animals used. In these two cases (14 and 31 days after the infection) there was a general infection of the organism. The bone-marrow contained *Brucella* on the fifth and sixth day, and regularly afterwards. Cultures from the bone-marrow were twice positive while those of the blood remained negative, and once *vice versa*. *Brucella* appeared in the blood on the ninth day. It seemed that some of the guinea-pigs might have resisted the infection altogether, or at least confined it to the lymphatic glands of the area of introduction (Nos. 10, 23). Guinea-pig No. 23, in which *Brucella* was only obtainable from the " mandibulo-pharyngeal " glands, did not possess agglutinins and complement-fixing bodies until the fifteenth day, while these usually appear as early as the ninth day.

2. *Conjunctival Infection.*

More or less the same results as in the previous group were obtained from guinea-pigs which had been infected in the conjunctiva. Positive cultures were obtained from the spleen after the first day, and from the liver after the third day. *Brucella* growth from the kidney occurred only once, after the seventeenth day, in which case there had been a general sepsis. Cultures made from the suprarenals were never followed by bacterial growth. Positive cultures, however, were obtained from the bone-marrow and blood after the seventh and eighth day respectively.

The fact that *Brucella* could be cultivated from the blood and " pharyngeo-mandibular " glands, and not from the other organs, of the last-mentioned case (No. 40), may be explained by the small amount of circulating bacteria (the first inoculation on solid culture medium from the broth-blood mixture was followed by no growth, while the second one gave a positive culture after the sixteenth day).

TABLE VII.

| After infection | 1 hour. | 5 hours. | 1 day. | 1 day. | 1½ days. | 2 days. | 3 days. | 3 days. | 4 days. | 5 days. | 5 days. | 6 days. | 6 days. | 8 days. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| "Mand. pharyng." glands ... | + | + 2 | | − | 1 | | | | | | | | | |
| Axillary glands ... ... ... | — | — | | — | — | — | | — | — | — | | — | | |
| Inguinal glands ... ... ... | — | — | | — | — | — | | — | — | — | | — | | |
| Mesenteric glands ... ... | — | — | — | — | | — | — | — | — | — | — | — | — | — |
| Bronchial glands ... ... | + | — | | — | — | — | | — | — | — | | — | | |
| Spleen ... ... ... ... | — | — | | — | — | 1 | — | — | — | — | | | — | — |
| Liver ... ... ... ... | — | — | — | — | — | — | — | 1 | — | — | | — | — | — |
| Kidney ... ... ... ... | — | — | — | — | — | — | — | — | — | — | 2 | — | — | — |
| Suprarenals ... ... ... | — | — | | — | — | — | | — | — | — | — | — | | — |
| Bone-marrow ... ... ... | — | — | — | — | | — | — | — | — | — | 1 | — | — | — |
| Blood ... ... ... ... | — | — | — | — | | — | — | — | — | — | | — | — | — |
| Agglutination titre ... ... | 0 | 0 | 0 | | | | 0 | | 0 | 0 | | 0 | 0 | 0 |
| Compl.-fixation index ... ... | 0 | 0 | 0 | | | | 0 | | 0 | 0 | | 0 | | 0 |
| No. ... ... ... ... | 1 | 2 | 3 | 4 | 5† *ps* | 6 | 7 | | 9 | 10 | 11† *pn* | 12 | 13 | 14 |

† = died. *ps* = pseudotuberculosis rodentium. *pn* = pneumonia.
In cases from which less than 10 colonies were cultivated the amount is given behind the plus sign.

TABLE VII—*Continued.*

| *Days after infection* | 9 | 9 | 9 | 10 | 10 | 11 | 12 | 14 | 15 | 17 | 22 | 22 | 31 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| "Mand. pharyng." glands ... ... | | — | | | +++ | | | | ++ | | | +++ | |
| Axillary glands ... ... ... | | — | | | — | | | | — | | | — | |
| Inguinal glands ... ... ... | | — | | | — | | | | — | | | +++ | |
| Mesenteric glands ... ... ... | — | — | $+_1$ | — | + | — | $+_1$ | + | — | ++ | + | $+_2$ | ++ |
| Bronchial glands ... ... ... | | — | | | | | | | — | | | | |
| Spleen ... ... ... ... ... | — | — | + | — | + | — | +++ | +++ | — | +++ | ++ | + | ++ |
| Liver ... ... ... ... ... | — | — | + | — | + | — | $+_1$ | + | — | + | $+_2$ | ++ | +++ |
| Kidney ... ... ... ... ... | — | — | — | — | — | — | $+_1$ | $+_2$ | — | — | — | — | + |
| Suprarenals ... ... ... ... | — | — | — | — | — | — | — | $+_1$ | — | — | — | + | — |
| Bone-marrow ... ... ... ... | — | — | + | — | + | — | + | $+_6$ | — | +* | $+_1$ | + | + |
| Blood ... ... ... ... ... | | | | | | | | | | | | | |
| Agglutination titre ... ... ... | 0 | 0 | 100 | 0 | 50 | 0 | 400 | 800 | 0 | 800 | 800 | 400 | 800 |
| Compl.-fixation index... ... ... | 0 | 0 | 6 | 0 | 7 | 0 | 10 | 10 | 0 | 9 | 10 | 10 | 9 |
| No. (continued) ... ... ... | 15 | 16 | 17† *pn* | 18 | 19 *pn* | 20 *pn* | 21 | 22 | 23 | 24 | 25 | 26 | 27 |

* = after 8 days negative, after 16 days positive. † = died. *ps* = pseudotuberculosis rodentium. *pn* = pneumonia.
In cases from which less than 10 colonies were cultivated the amount is given behind the plus sign.

TABLE VIII.

| *Days after infection* | 1 | 1 | 2 | 2 | 3 | 4 | 4 | 5 | 6 | 7 | 7 | 8 | 8 | 10 | 10 | 14 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| "Mand. pharyng." glands | | + | | — | | | — | | | | | | + | | | | |
| Axillary glands ... ... | | — | | — | | | — | | | | | | — | | | | |
| Inguinal glands ... ... | | — | | — | | | — | | | | | | — | | | | |
| Mesenteric glands ... ... | — | — | — | — | — | — | — | — | — | + | — | — | — | — | — | — | — |
| Spleen ... ... ... | $+_1$ | — | — | — | $+_3$ | + + | — | — | — | | + | $+_1$ | — | — | — | + + + | — |
| Liver... ... ... ... | — | — | — | — | + + | — | — | — | — | + | + | $+_1$ | — | — | — | $+_2$ | — |
| Kidney ... ... ... | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | |
| Suprarenals ... ... ... | | — | — | — | | — | — | — | | — | — | — | — | — | — | — | |
| Bone-marrow ... ... | — | — | — | — | — | — | — | — | — | + | — | $+_1$ | — | — | $+_1$ | + | + |
| Blood ... ... ... | — | — | — | — | — | — | — | — | — | | — | — | +* | | | + | + |
| Agglutination titre ... ... | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | (25) | 0 | | 100 | 800 | |
| Compl.-fixation index ... | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | 9 | 3 | 3 | | 3 | 10 | |
| No. (continued) ... ... | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37† | 38 | 39 | 40 | 41† | 42 | 43 | 44 |

* = after 8 days negative, after 16 days positive. † = died.
In cases from which less than 10 colonies were cultivated the amount is given behind the plus sign.

## Discussion and Conclusions.

Weigmann[9] performed similar experiments on guinea-pigs for the same purpose. In 16 cases he introduced, by means of an œsophageal tube, twice 10 c.c. of milk contaminated with *Brucella* into the stomach, and only detected the bacteria in the blood and organs of the animals which had been killed six weeks after infection. No comparison can be drawn between these findings and those obtained by the writer, for the passage of the micro-organisms from the mouth to the stomach is ignored by Weigmann's method. More complete and systematic investigations were carried out by Helms, Holm and ϕrskov[10] with mice, which according to a previous examination, they considered just as useful for this purpose as guinea-pigs. After having infected mice orally with enormous amounts of *Brucella* culture (one series with 100 million, three series with 1,000 million bacteria), they could cultivate *Brucella* after the first day quite regularly from the mesenteric, and especially from the maxillary glands, but not a single positive blood culture could be obtained. In these four series *Brucella* was found in the spleen for the first time after the sixth, eighth, tenth and eighteenth day respectively, and in the liver after the fourth, fourteenth and twenty-first day respectively. In one series it was not found at all in the liver. According to their obtained results, these Danish investigators concluded that infection occurs in the lymphatic system (micro-organisms first found in the regional lymphatic glands), and that after a growth of several days a more or less pronounced generalisation resulted only in a few cases from a distribution into the blood and organs along the centripetal lymphatic vessels. Initial infection of the blood was not found. These investigators therefore seek to explain this process by assuming that infection remains localised to the regional lymphatic apparatus of the alimentary tract, and subsequently becomes generalised after sufficient multiplication of the micro-organisms, when they finally escape with the lymph into the blood. These conclusions are, in general, not compatible with those that must be drawn from the author's researches, apart from the fact that relatively less virus and different test animals had been used.

Reviewing these observations concisely, we find the following: Three days after conjunctival infection in a *goat* the blood culture was positive. In a *horse* infected orally *Brucella* was also cultivated from the blood after three days; no culture was made earlier. In *dogs* the cultures were positive after one day, not only from the blood but also from the spleen, and in small quantities also from the regional lymphatic glands (madibulo-pharyngeal glands positive, intestinal glands negative). Three days after infection *Brucella* was found in spleen, liver, bone marrow, mesenteric and bifurcation glands (absent in the mandibulo-pharyngeal glands). The blood cultures were positive for

the first time one day, and for the second time 18 days, after the infection; afterwards they were repeatedly positive.

In the *guinea-pigs* many *Brucella* colonies were cultivated from the regional (" mandibulo-pharyngeal ") glands as early as one hour after oral infection. The micro-organisms were found in the spleen after one day, in the liver after three days, and eight days *post-infectionem* the blood culture was positive. Positive cultures from the mesenteric glands were only obtained at the time when there was a general blood infection.

In dogs and guinea-pigs it seems that there is rather a decrease of micro-organisms than a tendency towards distribution. Afterwards, however, general distribution occurs as a rule.

The great resistance of the kidneys and suprarenals is very noteworthy in all the cases of infection. It is possible to destroy this capacity of the kidney by poisoning it with uranyl nitrate.

The fact that *Brucella* are found regularly and so soon (one hour) in large quantities in the " mandibulo-pharyngeal " glands after oral or conjunctival infection, almost proves an immediate contamination of the regional lymphatic apparatus.

Positive cultures from the blood (in dog after one day, in horse and goat after three days), from the spleen (dog and guinea-pig after one day), from the liver (dog and guinea-pig after three days) and from the bone-marrow (dog after three days) can be better explained by assuming that the virus escapes from the " mandibulo-pharyngeal " glands into the blood stream by means of the blood capillaries, infecting these organs along this route, than by supposing that the micro-organisms enter into the blood from the draining lymphatics along the thoracic duct. As a rule, small amounts of *Brucella* culture were employed in the writer's experiments. It is improbable that such an amount of these bacilli could have been transported in such a short time from the region of the throat by means of the lymphatics, thus invading the blood and organs, without being caught in the intermediate lymphatic glands. That lymphatic transport from the regional lymphatic glands is of minor importance for the distribution of *Brucella* in the body is also supported by the fact that these bacilli were never cultivated from the cervical lymphatic glands during the first six days after infection, and very rarely after that period (Table VI).

It is also not impossible that the micro-organisms can be taken up directly by the blood capillaries from the area of introduction, quite independently of the lymphatic system. It is most probable, and also congruent with the author's findings, that the micro-organisms very soon after infection* are transported by venous blood from the regional lymphatic glands (*in casu* " mandibulo-pharyngeal " glands).

* As is indicated above (experiments in horses) *Brucella* can again appear in the blood amongst others subsequent to allergic reaction.

## References.

[1]Carpenter and Marriam. *J. Am. Med. Assoc.*, 1926, **87**, 1269.
[2]Amoss and Poston. *J. Am. Med. Assoc.*, 1929, **93**, 170; and 1930, **95**, 482.
[3]Van der Hoeden. *Tijdschr. Diergeneesk.*, 1932, **59**, 385.
[4]Soule. *1r Congrès Int. de Microbiol., Paris*, 1930, **1**, 606.
[5]Roots and Ridda. *Eesti Lomaarstlik Ringvaade*, 1932, **8**, 165.
[6]Van der Hoeden. *Zeitschr. Inf. Krankh. d. Haustiere*, 1932, **42**, 1.
[7]*Ibid. Tijdschr. Diergeneesk.*, 1932, **59**, 1383.
[8]Pflanz and Huddleson. *J. Am. Vet. Med. Assoc.*, 1931, **79**, 251.
[9]Weigmann. *Arch. f. Hyg. u. Bakter.*, 1929, **102**, 77.
[10]Helms, Holm and ϕrskov. *Z. f. Imm. forsch.*, 1932, **75**, 55.

# A STUDY OF THE RELATIVE EFFICACY OF PIGEON POX AND ATTENUATED FOWL POX VACCINES.

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The fact that birds which recover from a fowl pox infection possess a solid immunity against the disease led many investigators to believe that such an immunity could be produced by artificial inoculation. These investigations resulted in the development of several types of vaccines, which may broadly be divided into three classes:—

1. Dead fowl pox virus vaccines.
2. Attenuated fowl pox virus vaccines.
3. Biologically modified fowl pox virus vaccines.

An example of the first type of vaccine is that prepared by Hadley and B. A. Beach[1], later modified by J. R. Beach[2], which was prepared by heating fowl pox virus to 55° C. for one hour and was injected subcutaneously both for protective and curative purposes. Extensive field tests with this vaccine in the U.S.A.[3] showed that it had no immunising value.

The vaccines prepared by Johnson[4], Beach[5], and Kligler[6] represent the second type of vaccine. They differ somewhat in the manner of preparation but are fundamentally similar in that they contain living, if attenuated, fowl pox virus. It is generally agreed that these vaccines produce a long and perhaps lasting immunity, but investigators differ with regard to their safety. Beach[7] states that, notwithstanding the successful results of extensive field application of the living vaccines, it cannot be regarded as an entirely safe method. He records severe post-vaccination reaction, manifested by a drop in egg production as well as cases of death. Doyle[8] is of the opinion that vaccination with active attenuated fowl pox virus vaccine is dangerous and justified only in exceptional circumstances.

The vaccines prepared by Zwick[9], Doyle[8] and others belong to the third type. This vaccine consists of pigeon pox virus, or of a fowl pox virus modified by passage through pigeons. It produces only a mild reaction in chickens and can be used directly without further treatment. No unfavourable results, such as secondary lesions, drop in egg production, etc., have been recorded with the use of this vaccine; investigators differ, however, regarding the duration of the immunity produced. Doyle[8] maintains that the treated birds are solidly immunised against natural infection for a period of about one year and show considerable resistance to artificial inoculation. Lehage[10] reports that birds

vaccinated with pigeon pox virus were completely immunised against natural as well as artificial inoculation. Dalling[11], on the other hand, is of the opinion that the period of immunity is not over four months.

*Fowl Pox in Palestine.*

Fowl pox, in its manifold forms, is a highly prevalent disease in Palestine. It makes its appearance early in winter and spring, causing a reduction in egg production when eggs are highly priced, loss of condition, and at times heavy mortality. It is, therefore, a disease of considerable economic importance to the country.

Until three years ago no vaccines were available in this country for prophylactic purposes, and the disease was mainly combated through hygienic means and by individual treatment of infected birds. Subsequently De Blieck Antidiphtherin was imported from Holland and used quite extensively, with variable results. Recently Beach's Fowl Pox Vaccine and Kligler's Fowl Pox Vaccine and Pigeon Pox Vaccine have also been used. The use of different vaccines offered an opportunity to compare their relative merits under field conditions, and it soon became apparent that before any one vaccine could justly be recommended additional controlled experiments bearing on the efficacy of the different kinds, their safety, and the relative duration of the immunity conferred, were required.

EXPERIMENTAL WORK.

*Experiment I.*—The purpose of this experiment was to determine the relative value of a standardised, phenolised, fowl pox virus as prepared by Kligler, and a fresh pigeon vaccine.

*Standardisation of the Phenolised Fowl Pox Virus Vaccine.*

A 1 : 10 emulsion of fresh fowl pox virus was prepared in 0·2 per cent. phenolised saline solution and placed in the ice box. After storage for from two weeks to one month the stock solution was diluted to 1 : 1,000, 1 : 10,000, 1 : 20,000, 1 : 40,000, 1 : 80,000, and a drop (0·05 c.c.) of each dilution was inoculated on different points of the comb of a susceptible bird. The final examination of the bird is made ten to twelve days after the inoculation. The following is a typical protocol :—

*Results of Virulence Test.*

| | |
|---|---|
| 1 : 1,000 | 12 vesicles |
| 1 : 10,000 | 3 ,, |
| 1 : 20,000 | 2 ,, |
| 1 : 40,000 | negative |
| 1 : 80,000 | ,, |

On the basis of this test the stock virus emulsion was diluted to 1 : 10,000 and used as vaccine. The vaccine is applied by rubbing a drop into the feather follicles of the thigh after the removal of some feathers.

*Preparation of Pigeon Pox Vaccine.*

Pigeons are inoculated on the scarified breast and back with pigeon pox virus. Nine to twelve days after the inoculation the scabs are removed, dried in a dessicator, and powdered. Just before use, 500 mgm. of the dry virus are diluted in 70 c.c. of 50 per cent. glycerinated saline solution. After thorough mixing the vaccine is applied in the same manner as the fowl pox virus vaccine.

*Birds and Premises.*

Two flocks of healthy pullets ranging in age from two to four months were vaccinated. The two flocks were housed in separate quarters far removed from one another. In each group ten unvaccinated birds were left untreated as controls. The results are shown in Table I.

TABLE I.

RESULTS OF VACCINATING CHICKENS WITH DIFFERENT TYPES OF VACCINE.

(*a*) *Phenolised Standardised Fowl Pox Virus.*

Vaccinated June 17th, 1932.

| | *Dates of Examination.* | | | | |
|---|---|---|---|---|---|
| | *June* 22. | *July* 1. | *July* 15. | *July* 30. | *Aug.* 22. |
| Number of birds ... ... | 49 | 49 | 47 | 47 | 47 |
| Per cent. with strong takes ... | 0 | 47 | 27·6 | 0 | 0 |
| Per cent. with good takes ... | 100 | 53 | 23·4 | 8·5 | 0 |
| Per cent. with takes off ... | 0 | 0 | 49 | 91·5 | 100 |
| Per cent. with secondary lesions | 0 | 18·2 | 19·9 | 2·1 | 0 |
| Per cent. infections in controls | 0 | 0 | 70 | 30 | 0 |

(*b*) *Pigeon Pox Virus.*

| | *Dates of Examination.* | | |
|---|---|---|---|
| | *June* 22. | *July* 1. | *July* 15. |
| Number of birds ... ... | 256 | 256 | 256 |
| Per cent. with strong takes ... | 0 | 0 | 0 |
| Per cent. with good takes ... | 100 | 80 | 0 |
| Per cent. with takes off ... | 0 | 80 | 100 |
| Per cent. with secondary lesions | 0 | 0 | 0 |
| Per cent. infections in control | 0 | 0 | 0 |

It can be noted from Table I that :—

(*a*) The virus was viable and active in both vaccines as indicated by the percentage of takes.

(*b*) That in the case of the pigeon pox vaccine the takes scaled off two weeks following vaccination, while in that of the phenolised fowl pox virus the scaling began during the third week following vaccination and the scabs were off at the end of the fourth week.

(*c*) That in the case of phenolised fowl pox virus the local reaction as manifested by the size and character of the takes was much stronger than in the pigeon pox group.

(*d*) That birds vaccinated with phenolised fowl pox virus constituted a source of infection to unvaccinated susceptible birds (controls).

(*e*) That in the fowl pox virus vaccine secondary lesions and mouth lesions developed in about 20 per cent. of the birds.

(*f*) In neither group was there any mortality, the birds being fully recovered within four to six weeks after vaccination.

*Experiment II.*—The purpose of this experiment was to extend the observations recorded above and to compare the effect of the vaccines used in the above experiment with that prepared according to Beach.[5]

*Preparation of Beach Vaccine.*—One gram of fresh fowl pox virus scabs was emulsified in 9 c.c. of 0·5 per cent. phenolised physiological salt solution. This stock solution was used shortly after preparation and was diluted immediately before use in the proportion of 1 : 50, one drop being applied to the feather follicles of the thigh by means of a stiff brush. The brushing was harder than employed by Beach and apparently gave heavier takes than would have been obtained by milder application.

*Birds and Premises.*—A flock of 187 birds was divided into three equal groups with respect to number and age. These chickens were of late hatch and probably not as healthy as earlier hatches. The ages of the pullets ranged from three to five months. They were all housed in a large brooder house which was divided by means of chicken netting into five parts. The groups to be vaccinated were so placed that between each vaccinated group was a flock of unvaccinated birds. Unvaccinated control birds were left in each vaccinated group. The results are recorded in Table II.

TABLE II.

RESULTS OF VACCINATING CHICKENS WITH DIFFERENT TYPES OF VACCINE.

(*a*) *Pigeon Pox Vaccine.*

Vaccinated October 31st, 1932.

| | *Dates of Examinations.* | | | |
|---|---|---|---|---|
| | *Nov.* 8. | *Nov.* 16. | *Nov.* 24. | *Dec.* 5. |
| Number of birds ... ... ... | 55 | 54 | 54 | 53 |
| Per cent. with strong takes ... ... | 22·8 | 0 | 0 | 0 |
| Per cent. with good takes ... ... | 53·0 | 0 | 0 | 0 |
| Per cent. with slight takes ... ... | 14·5 | 5·5 | 0 | 0 |
| Per cent. with no takes or takes off ... | 9·9* | 94·5 | 100 | 100 |
| Per cent. with comb lesions ... ... | 0 | 0 | 0 | 0 |
| Per cent. with mouth lesions ... ... | 0 | 0 | 0 | 0 |
| Per cent. with comb and mouth lesions | 0 | 0 | 0 | 0 |
| Total per cent. of secondary infection | 0 | 0 | 0 | 0 |
| *Controls.* | | | | |
| Per cent. with comb lesions ... ... | 0 | 0 | 0 | 16·6 |
| Per cent. with mouth lesions ... ... | 0 | 0 | 0 | 16·6 |
| Per cent. with comb and mouth lesions | 0 | 0 | 0 | 0 |
| Total per cent. of infection ... ... | 0 | 0 | 0 | 32·2 |

* No takes.

(*b*) *Fowl Pox Virus and Standardised Vaccine.*
Vaccinated October 31st, 1932.

| | *Dates of Examination.* | | | |
|---|---|---|---|---|
| | *Nov.* 8. | *Nov.* 16. | *Nov.* 24. | *Dec.* 5. |
| Number of birds ... ... ... | 67 | 63 | 62 | 60 |
| Per cent. with strong takes ... ... | 0 | 46 | 28 | 0 |
| Per cent. with good takes ... ... | 68·6 | 47·1 | 16·6 | 1·6 |
| Per cent. with slight takes ... ... | 31·4 | 7 | 29 | 0 |
| Per cent. with no takes or takes off ... | 0 | 0 | 26·4 | 98·4 |
| Per cent. with comb lesions ... ... | 0 | 9 | 30·8 | 6·6 |
| Per cent. with mouth lesions ... ... | 0 | 10·4 | 14·5 | 3·2 |
| Per cent. with comb and mouth lesions | 0 | 3 | 12·9 | 1·6 |
| Total per cent. of secondary infection | 0 | 22·4 | 58·2 | 11·4 |
| *Controls.* | | | | |
| Per cent. with comb lesions ... ... | 0 | 33·3 | 33·3 | 66·6 |
| Per cent. with mouth lesions ... ... | 0 | 0 | 22·2 | 11·1 |
| Per cent. with comb and mouth lesions | 0 | 0 | 22·2 | 11·1 |
| Total per cent. of infection ... ... | 0 | 33·3 | 77·7 | 88·8 |

(*c*) *Fowl Pox Virus Vaccine, Beach.*
Vaccinated October 31st, 1932.

| | *Dates of Examination.* | | | |
|---|---|---|---|---|
| | *Nov.* 8. | *Nov.* 16. | *Nov.* 24. | *Dec.* 5. |
| Number of birds ... ... ... | 65 | 63 | 62 | 56 |
| Per cent. with strong takes ... ... | 0 | 44·4 | 35·4 | 5 |
| Per cent. with good takes ... ... | 95·3 | 55·6 | 39 | 8·4 |
| Per cent. with slight takes ... ... | 4·7 | 0 | 14·5 | 0 |
| Per cent. with no takes or takes off ... | 0 | 0 | 21·5 | 86·6 |
| Per cent. with comb lesions ... ... | 0 | 11·1 | 14·5 | 5 |
| Per cent. with mouth lesions ... ... | 0 | 23·8 | 24·1 | 8·4 |
| Per cent. with comb and mouth lesions | 0 | 14·2 | 32·2 | 6·7 |
| Total per cent. of secondary infection | 0 | 49·1 | 70·8 | 20·1 |
| *Controls.* | | | | |
| Per cent. with comb lesions ... ... | 0 | 22·2 | 44·4 | 22·2 |
| Per cent. with mouth lesions ... ... | 0 | 22·2 | 11·1 | 33·3 |
| Per cent. with comb and mouth lesions | 0 | 0 | 11·1 | 0 |
| Total per cent. of infection ... ... | 0 | 44·4 | 66·6 | 55·5 |

It will be noted from Table II that the results of this experiment correspond in general with those of the previous one. The pigeon pox takes disappeared after two weeks, those of the fowl pox groups only after four to five weeks. There was a slight difference with regard to secondary lesions in favour of the standardised vaccine, as compared with that prepared by Beach. Four birds out of the 67 vaccinated with the standardised vaccine, and five out of the 66 vaccinated with Beach vaccine, were killed because of the severe pox lesions. Other fatalities had no relation to the vaccination.

*Immunity Tests.*

*Resistance to Artificial Inoculation.*—In order to ascertain the relative resistance of birds treated with different vaccines to artificial inoculation, several chickens from the above experiments

were inoculated together with controls, with active fowl pox virus at different periods after vaccination. The freshly prepared fowl pox virus was inoculated in different dilutions on the comb and wattles and the result recorded 14 to 21 days later. The data are tabulated in Table III.

TABLE III.

RESISTANCE OF IMMUNISED CHICKENS TO ARTIFICIAL INFECTIONS.

*Control.*

| *Dilution of Pox Virus.* | *Group I.* | *Group II.* | *Group III.* | *Group IV.* |
|---|---|---|---|---|
| 1 : 1,000 | +++ | +++ | +++ | +++ |
| 1 : 10,000 | +++ | +++ | +++ | +++ |
| 1 : 20,000 | ++ | +++ | +++ | +++ |
| 1 : 40,000 | ++ | +++ | +++ | ++ |
| 1 : 80,000 | + | +++ | ++ | ++ |

Immunised with

*Pigeon Pox Virus.*

Vaccinated June 17th, 1932. Retested after :—

| *Dilution of Pox Virus.* | *2 months.* *2 birds.* | *3 months.* *5 birds.* | *5 months.* *3 birds.* | *6 months.* *3 birds.* |
|---|---|---|---|---|
| 1 : 1,000 | + + | + ++ + ++ ± | — + — | + — — |
| 1 : 10,000 | ± ± | + + ± ++ — | — ± — | — — — |
| 1 : 20,000 | ± — | ± ± ± + — | — — — | — — — |
| 1 : 40,000 | ± — | — — — ± — | — — — | — — — |
| 1 : 80,000 | — — | — — — — — | — — — | — — — |

Immunised with

*Fowl Pox Virus.*

Vaccinated June 17th, 1932. Retested after :—

| *Dilution of Pox Virus.* | *2 months.* *2 birds.* | *3 months.* *4 birds.* | *5 months.* *3 birds.* | *6 months.* *3 birds.* |
|---|---|---|---|---|
| 1 : 1,000 | ± — | ± ± ± — | — — — | — — — |
| 1 : 10,000 | — — | — ± — — | — — — | — — — |
| 1 : 20,000 | — — | — — — — | — — — | — — — |
| 1 : 40,000 | — — | — — — — | — — — | — — — |
| 1 : 80,000 | — — | — — — — | — — — | — — — |

Retested after 2½ months.

| *Dilution of Pox Virus.* | *Control.* *2 birds.* | *Pigeon Pox Immune.* *Vac. Oct. 31st, 1932.* *2½ months, 3 birds.* | *Fowl Pox Immune.* *Vac. Oct. 31st, 1932.* *2½ months, 4 birds.* |
|---|---|---|---|
| 1 : 1,000 | +++ +++ | ±— ±— — | — — — — |
| 1 : 10,000 | +++ +++ | — — — | — — — — |
| 1 : 20,000 | +± +± | — — — | — — — — |
| 1 : 40,000 | | — — — | — — — — |
| 1 : 80,000 | | — — — | — — — — |

+++ = over 30 vesicles. ++ = over 20 vesicles. + = over 10 vesicles.
± = 1 to 5 vesicles. — = negative.

Although the number of birds retested was rather small, it can be noted from this table that, while the birds vaccinated with pigeon pox virus possess a certain degree of immunity to artificial inoculation, the immunity is not as complete as in those chickens treated with fowl pox virus. In all the cases the lesions were mild and healed more rapidly than in the control. It is interesting that birds of Experiment 1, tested six months after vaccination, possessed a greater resistance to the inoculation than those imunised later.

*Resistance to Natural Infection.*—In order to ascertain the relative resistance of the birds treated with the different vaccines to natural infection the following experiments were carried out.

*Experiment A.*—On December 13th the birds vaccinated on October 31st were removed to a large laying house and susceptible birds were placed among them. Beginning January 12th, several cases of pox were noted among the susceptible birds. On January 27th all the birds in the laying house were examined and the following noted :—

(*a*) Over 50 per cent. of the susceptible birds had severe comb and mouth lesions.

(*b*) Six out of 53 birds vaccinated with pigeon pox vaccine had mild comb lesions.

(*c*) Of the birds vaccinated with the fowl pox vaccine none had any lesions.

*Experiment B.*—Two pigeon pox immune birds vaccinated six months previously, and four birds vaccinated two and a half months previously, were placed together with five infected birds in a roofless open shed. During the period of observation, which lasted 29 days, the birds were exposed to bad weather conditions, low temperature, winds and rain. Out of the two birds vaccinated six months previously, one developed a mild diphtheritic lesion on the twelfth day, and the other remained negative. Out of the four vaccinated two months previously, three became infected six, 13, and 16 days after the start of the experiment. The bird which became positive on the sixth day was probably in the incubation period of the disease before the experiment started. The lesions were mild and tended to heal rapidly.

## Discussion.

The results of the above experiments warrant certain tentative deductions. It is apparent that the pigeon vaccine produces mild and rapidly healing lesions, while the attenuated fowl pox virus vaccines produce more severe lesions which last a longer time, and which may in a greater or lesser percentage of birds be accompanied by secondary lesions on the comb or in the mouth. The use of pigeon pox vaccine entails, therefore, no risk of secondary infection, while the use of fowl pox presents an element of risk which in healthy flocks is relatively small. These results were confirmed by observations in the field. In flocks where standardised attenuated fowl pox virus vaccine was used secondary lesions appeared, but the mortality, if any, which could be attributed directly to

vaccination was low. In one flock, vaccinated with phenolised vaccine, which was suffering from spirochetosis and in which some of the birds showed symptoms of A avitaminosis, there was a considerable mortality after the vaccination, but it was not clear that any of the mortality was attributable to the vaccine lesions. It seems that in flocks in poor condition the use of attenuated fowl pox vaccine is associated with appreciable risk; in healthy birds, however, secondary lesions, if they appear, are mild in character and easily eliminated by treatment.

Regarding the degree and duration of the resulting immunity, it seems clear that the pigeon pox vaccine is less effective. In large scale vaccination about 10 per cent. of the birds do not develop takes at all and are consequently devoid of any immunity. Those that are immunised resist low doses of virus or mild exposure, but heavier doses or more intense exposure result in fowl pox infection. It appears, however, that birds vaccinated with pigeon pox virus slowly acquire an increased immunity by exposure to infection which at all events is mild in character. It is not possible as yet to say what effect this lessened immunity might have on the epidemicity of fowl pox. In five out of 60 settlements in which pigeon pox vaccine was used this year outbreaks of fowl pox occurred four to six months after vaccination, but in no case were more than 10 per cent. of the birds affected. This indicates a reasonably satisfactory flock immunity. The present indications are, therefore, in favour of pigeon pox vaccines, particularly in weak flocks, with the precautionary measure, perhaps, of semi-annual revaccination in infected areas, should that prove practicable. Experiments are in progress on a more generally satisfactory method of immunisation.

## Conclusions.

1. No risk of secondary lesions is involved in the use of pigeon pox vaccine.

2. The risk involved in the use of fowl pox vaccine is relatively small in the case of healthy birds, but may be more serious in an unhealthy flock.

3. Pigeon pox vaccine produces mild, promptly healing lesions without secondary involvement.

4. Attenuated fowl pox vaccine produces severe lesions, of longer duration, and often associated with secondary lesions in the mouth and comb.

5. The duration as well as the degree of immunity produced by attenuated fowl pox vaccines is greater than that produced by the pigeon pox vaccine. In the latter case the immunity is less solid and approximately 10 per cent. of the birds remain unimmunised.

## References.

[1]Hadley, F. B., and Beach, B. A. Proc. 15th Ann. Meet. A.V.M.A., 1913, 704-712.
[2]Beach, J. R. Jour. A.V.M.A., 1920, lviii, 301-310.
[3]Boerner, F., and Stubbs, E. L. Jour. A.V.M.A., 1921, ix, 83-91.
[4]Johnson, W. T. Jour. A.V.M.A., 1927, lxxi, 750-763.
[5]Beach, J. R. Hilgardia, 1927, iii, 41-97.
[6]Kligler, I. J. Brit. Jour. Exp. Path., 1930, xi, 10-13.
[7]Beach, J. R. Cal. Agr. Est. Serv. Cir. 8, June, 1930, 6-23.
[8]Doyle, T. M., and Minett, F. C. Jour. Comp. Path. & Therap., 1927, iv, 247-266.
[9]Zwick, W., Seigfried, O., and Schaaf, J. Zeit. f. Krankheiten, 1928, **34**, 300.
[10]Lehage, J. Am. Vet. Med., 1928, **73**, 297.
[11]Dalling, T., and Wanrock, G. H. Notes on Investigations into Some Poultry Diseases. Wellcome Phys. Res. Lib., 3-4.

# EDITORIAL.

## SWINE FEVER AND PARATYPHOID IN PIGS.

Salmon and Smith (1885) showed that in fatal cases of hog cholera an organism, which they named *Bacillus choleræ suis*, could be cultivated from the blood and organs, and they also showed that swine could be infected by the injection of pure cultures of this organism or by feeding with cultures. *Post-mortem* examination of pigs so infected revealed lesions similar to those found in animals that had contracted the disease naturally, and they were able to recover the bacillus from the organs.

As the results of these observations and experiments, which were confirmed with regard to the disease in Europe, it soon came to be generally assumed that the ætiology of hog cholera and swine fever had been definitely settled.

Soon afterwards in the United States attempts were made to deal with the disease by employing a vaccine or serum, the vaccine being an artificial culture of the *B. choleræ suis*, while the serum was obtained from pigs immunised with the same organism. The vaccines were prepared by attenuating the organism or by extracting from it substances which were supposed to be concerned in the production of immunity. In the opinion of the Bureau of Animal Industry none of these vaccines or serums gave satisfactory results in practice, and for 20 years after the discovery of the supposed cause of the disease little or no progress was made in combating it. It may be added that in many cases the immediate result of the vaccination was a fatal attack of what appeared to be, and was assumed to be, hog cholera.

In the course of experiments it was observed that pigs that became ill and died from the vaccination were not a source of danger to healthy animals kept with them, and this fact appeared to be very remarkable since one of the most striking characteristics of hog cholera is its extraordinary capacity for spreading by contagion.

Complementary to these discoveries, it was found that the disease set up by the injection of hog cholera blood, in marked contrast to that produced by the cultures, had the same contagious character as the natural disease, and hogs that survived this inoculation were completely immune against the natural disease. It was these investigations which suggested that the cause of hog cholera might be some ultra-visible organism present in the blood and led to the discovery of the virus by de Schweinitz and Dorset (1903).

The discovery of the virus as the true cause of the disease left unexplained the great frequency, amounting almost to constancy, with which the *B. suipestifer* could be cultivated in a state of purity from the blood and organs in cases of swine fever. On this

subject opinion has been much divided, but now appears to be approaching unanimity. The points in dispute have been (1) the respective rôles of the virus and the bacillus in the causation of the symptoms and lesions, and (2) the biological or epidemiological characters of the bacillus. The first question includes both symptoms and lesions but only the latter need be here considered. Two views have been put forward regarding the ætiology of the lesions, *viz.* : (*a*) that the virus is alone responsible, and (*b*) that the responsibility in cases of the natural disease is divided.

Unfortunately, the fact that the virus is ultra-visible and uncultivable excludes methods of research regarding pathogenesis that are available in diseases caused by visible organisms, but, in compensation for this disability, the filterability of the virus is a great advantage because of the readiness with which it enables one to obtain the pure virus. An important fact in this connection is that, although the virus is the constant factor in the causation of the disease, in the great majority of natural infections the bacillus is also demonstrably present. The natural disease is thus generally a mixed infection of virus and bacilli. In cases of experimental infection by inoculation with the virus the results are a little different, for it would appear that in such cases it may be impossible to demonstrate the bacilli in pigs that have died or been killed within a few days after inoculation. These cases are important inasmuch as they appear to prove that the virus by itself can cause fatal effects without the concurrence of the bacilli. It must be pointed out, however, that the point is not whether the virus alone can kill but whether it is capable, by itself, of producing what is called the pathological picture of natural cases in respect of lesions.

Clear evidence regarding the capacity of the pure virus for causing lesions is rarely obtainable in natural cases for the reason already mentioned, *viz.*, the fact that in natural cases in the field the infection, except in the initial stage, is a mixed one. More was to be expected from observation of the nature and extent of the lesions resulting from inoculation with the pure filtrate virus. The evidence on the point is not very extensive, but it indicates that in the very acute cases the diphtheritic and ulcerative lesions of the intestine, which are so constant in cases of natural infection, may be absent. The pathological alterations present in these cases resemble those which are found in pigs that have suffered from the disease in what is called the peracute form.

More briefly stated, the facts in this connection indicate that extensive lesions of diphtheresis and ulceration of the mucous membrane of the large bowel in swine fever are mainly, if not entirely, to be laid to the charge of the *B. suipestifer* and not of the virus.

It is true that in some experiments with pure virus as the

infecting agent slight diphtheritic inflammation has been found in the intestine although a search for the presence of the bacillus had a negative result. It may fairly be said, however, that in some of the experiments and observations of that kind the methods employed to detect the presence of the *B. suipestifer* in the bowel contents and the lesions may not have been adequate for the purpose.

In considering in the next place the pathogenic power of the *B. suipestifer,* it is to be noted that, with the possible exception of certain lesions bordering on the microscopic and affecting only the histology of the tissues and organs, it is often impossible by *post-mortem* or bacteriological examination to distinguish between a natural case resulting from infection by contact and an experimental case caused by feeding with pure artificial culture of the bacillus.

All the recognised stages of the natural disease have been observed in pigs fed or inoculated with artificial cultures of the bacilli, including at the one end hyperacute cases without any gross lesions except acute inflammation of the mucous membrane of the stomach and intestines, and at the other end cases with extensive, obviously chronic, inflammation and ulceration of the wall of the large intestine.

Since the virus of swine fever is ultra-visible and uncultivable, neither its presence nor its absence can be proved except by experimental methods, but a search for it in the body is greatly facilitated by its filterability. By taking advantage of that, it was first possible to study what may be called the pure disease, uncomplicated by the effects of any other pathogenic agent. That is now what is called the virus disease.

There can be no doubt that, in all cases in which swine contract the disease naturally, it is at the outset pure virus disease in the sense that the virus alone is responsible for the observed departures from the normal. The duration of this virus stage of the disease, however, is usually a short one, and therefore little opportunity is afforded for studying the pure virus disease in field cases. Consequently nearly all that is known about the initial stage has been obtained by the study of cases experimentally infected with the pure virus, that is, with filtrate.

As bearing on the question of the respective parts played by the virus and the *B. suipestifer* in natural cases of the disease, a comparison of the effects produced by experimental infection with filtrate and with pure culture of the bacillus is instructive.

In the period between the discovery of the bacillus and the discovery of the virus much information was obtained in experiments carried out with the bacillus in the belief that it was the veritable cause of the disease, and, in retrospect, it seems remarkable that for over 20 years none of those who studied the disease set up by experimental infection with artificial cultures of the bacillus observed anything to suggest that it differed in any way

from the natural disease contracted by contagion. That by itself affords proof that if there is an independent enteritis of the pig caused by the *B. suipestifer* it must be exceedingly difficult to distinguish it, in respect of its lesions, from natural swine fever.

Apparently the effects of infection with filtrate vary to some extent with the dose, but very minute amounts suffice, as is no doubt the case also in the natural spread of the disease. When a series of pigs are inoculated with the virus and killed at different short intervals afterwards it is possible to ascertain the length of time for which the virus operates alone without the help of any other organism, and it has been found in this way, as already stated, that the period is usually short when measured from the moment when the first symptoms of illness are exhibited. It has been estimated that in about 80 per cent. of cases infected in that way the secondary invasion begins before the pig dies or becomes seriously ill. It is a pretty safe inference that also in cases contracted naturally by contagion the disease is usually a mixed infection throughout the whole period of visible illness.

Turning next to consider more closely the respective parts played by the virus and the *B. suipestifer* in the production of the lesions, it appears to have been established by experiments that, except in a small proportion of cases, there are no constant differences between the lesions in cases of experimental infection with pure virus and those found in cases of natural infection, in animals that have died from the disease or been killed when visibly ill. That, however, does not by itself enable one to apportion the blame for the lesions between the virus and the bacilli, since, except in very acute cases, both are usually present throughout the course of the visible illness.

The present writer agrees with those who think it probable that the *B. suipestifer* is mainly or entirely responsible for the visible structural alterations in the bowel, especially the acute, diffuse, diphtheritic inflammation and the formation of definite ulcers in the cæcum and colon. That seems to be indicated by the fact that such lesions, indistinguishable from those usually found in natural cases of the disease, are equally common in pigs infected with artificial cultures of the bacillus—that is, cases in which the virus has played no part.

On the other hand, recent experiments by Waldmann and others indicate that in the hyperacute cases the fever and vascular disturbances are probably the result of multiplication of the virus in the body and its great abundance in the blood.

The view that appears to explain the almost constant simultaneous presence of both the virus and the bacilli is, that in all parts of the world where pigs are found the *B. suipestifer* is present in the environment, most probably as the result of fæcal contamination. This view involves the further supposition that healthy pigs, in consequence of a natural resistance, are not affected by the presence of the bacilli in the intestine in

ordinary circumstances. Further, it must be supposed that infection of a healthy pig with the pure virus weakens this resistance and thus enables the bacilli to begin to multiply and invade the body. This conclusion has had the support of many observations, but it is only lately that it has been proved by the discovery that in about 80 per cent. of pigs infected by the pure virus the *B. suipestifer* appears in the body of the pig in numbers that make the demonstration of its presence easy. The recorded cases in which animals infected with the pure virus have died without invasion by the bacillus cannot seriously be advanced against the above conclusion, and it is probable that the proportion might be further reduced if in all such cases more searching attempts were made to find the bacillus, particularly in the lymphatic glands of the large intestine, which is a predilection site of the organism.

At any rate, no one has put forward any other suggestion that will explain the fact that, almost anywhere, by taking a pig at random from a healthy stock and infecting it with the pure virus, the *post-mortem* when the pig dies will show the presence of the *B. suipestifer* as well as of the virus. As already stated, naturally contracted swine fever is a mixed infection in the sense that two organisms—the virus and the bacillus—co-operate in producing the lesions, but not in the sense of a double separate introduction of these organisms through contagion.

The fact that *B. suipestifer* can, by itself, be highly pathogenic for pigs, and cause lesions almost identical in appearance with those found in cases of swine fever naturally contracted, has been one of the chief stumbling blocks in diagnosis.

The assumption that paratyphoid bacilli, normally present in the bowel of pigs, may suddenly become highly virulent, invade the blood and organs, cause death, and serve as the starting point of an outbreak, is now discredited. It is diminished resistance on the part of the pig and not increased virulence of the *B. suipestifer* that accounts for the sudden invasion by the organism.

The cultivation of the *B. suipestifer* or any other member of the paratyphoid group from the carcase of a pig, and demonstration of the fact that the organism thus obtained is pathogenic for pigs, is very far from proving that the case in question was not one of swine fever.

In this connection the only safe rule is not to diagnose paratyphoid in pigs until the non-existence of swine fever in the herd has been proved.

It is not to be inferred from what has just been said that a search for the ultra-visible virus should be a routine proceeding in the diagnosis of swine fever. On the contrary, we agree with those who think that the procedure which for many years has been prescribed for inspectors under the Ministry of Agriculture, is capable of conducting an experienced inspector to a correct diagnosis in the immense majority of cases. The elucidation of the exceptional cases is work for the laboratory.

*Is there an Infectious Enteritis of Pigs Distinct from Swine Fever?*—As the adjective "infectious" lacks precision, it may be as well to say that the point about which information is asked is, whether infectious enteritis or necrotic enteritis, or paratyphoid in pigs, is a disease which not only simulates swine fever in respect of course, symptoms and lesions, but is also, like the latter disease, contagious. Apparently on the available evidence that question must have a negative answer.

The notion that the presence of diphtheritic inflammation and ulcerative lesions in the large intestine of the pig is characteristic of swine paratyphoid is erroneous. These lesions are commonly found both in that diseased condition and in swine fever, and that is mainly responsible for the difficulty in distinguishing between them by simple *post-mortem* examination. On this subject we commend to all who are interested the opinions of the eminent German authors which will be found elsewhere in this number.

The paratyphoid of pigs which in recent years has been the cause of serious losses in the large breeding herds on the Continent appears to be almost entirely a disease of sucking pigs or pigs under three months old, and even in such young animals to be rarely observed except under bad hygienic conditions, including overcrowding and defective feeding. It is very desirable that it should not be confounded with swine fever, as obviously it does not call for notification or interference with the movement of the diseased animals.

The present difference of opinion with regard to the true nature of the so-called paratyphoid of pigs recalls a similar dispute with regard to the nature and ætiology of pneumonia of the pig, the so-called *Schweineseuche*, sometimes termed swine plague in this country and the United States. For a time it was very stoutly maintained that this was an independent disease of the pig, and it was believed that it could even attain epizoötic dimensions among pigs, quite independently of swine fever. It was not denied by anyone that the bipolar-staining ovoid organism found in the hepatised lung is the actual cause of the pneumonia, but it is now generally recognised that the ovoid bacteria are normally found in the air passages of pigs, and that it is the action of the virus of swine fever that diminishes the normal resistance of the body and thus enables the ovoid bacteria to multiply and cause pneumonia.

No one can fail to see the strict parallelism between the ordinary cases of swine fever in which the virus and the *B. suipestifer* are associated in causing the lesions, and the rarer cases in which the association is between the virus and the bipolar bacteria. It is a fact, however, that both the *B. suipestifer* and the *B. suiseptica* may be active in the same pig in cases of swine fever.

It would be improper for anyone holding the views that have

just been expressed to ignore the fact that directly contrary opinions are to be found in the literature on the subject. The following is an example :—

In 1928 Murray, Biester, Purwin and McNutt* published the result of their studies in " Infectious Enteritis of Swine " which they had conducted at the State College of Iowa.

In the first paragraph of this report the authors stated that they were convinced " that the primary cause of this disease is the *Salmonella suipestifer.*"

The next following sentence is : " Other organisms which have been incriminated by other investigators are, in our opinion, merely secondary invaders, or play no rôle at all in the ætiology of the disease." The " other investigators " must have included Salmon and his co-workers, but no one would have inferred from this sentence that Salmon was the real discoverer of the bacillus now put forward as the primary cause of infectious enteritis.

The findings and conclusions in the report were based upon a detailed study of : " Twenty cases of infectious enteritis [so-called necrotic enteritis] obtained from the field, forty-three cases of the same disease induced experimentally, material from two cases of human typhoid fever, material from three cases of human *Balantidium colitis,* and button ulcers from one case of hog cholera."

The natural cases of infectious enteritis which served as the basis of the researches were field cases, that is, they were obtained from a herd or herds in which pigs were ill and dying from a disease in which, as was afterwards proved, the principal lesions affected the large intestine, and which, from the authors' own account, were apparently not distinguishable from the lesions in the same position in cases of hog cholera.

It must also be noted that presumably (though not expressly stated) this infectious enteritis was spreading by contagion in the herd or herds from which the field cases were obtained, for diseased pigs for study were obtainable in lots of four at a time.

No detailed account is given of the individual cases except concerning the temperature reactions in pigs fed with cultures of the " suipestifer-type organism " isolated from field cases. Space might have been spared if it had been simply stated that in this respect, and also in respect of the course of the disease and the lesions, the results were similar to those described by Salmon and other subsequent investigators who worked with the same organism.

The possibility that the diseased field cases which formed the basis of the study were affected with swine fever is not seriously discussed in the paper, but it is said that the physical condition and a knowledge of the source of the pigs used for experimental purposes afforded reasonable assurance that they

* Journal A.V.M.A., Vol. 72, 1928, pp. 35-65.

were healthy pigs. It is stated that when healthy pigs were obtained for the purpose of producing the disease experimentally some were kept over for a varying period without feeding culture, and in a manner served as checks, since no sickness nor fatalities occurred in such animals. This, however, can hardly mean that the supposedly healthy pigs intended for experiment were kept over in contact with the diseased field cases. Presumably what is meant is, that the healthy pigs in contact with the pigs infected with culture remained healthy, which is exactly what one would have expected since.

In a subsequent article appearing in the same journal (pp. 1003-1022), the authors deal with the lesions found in pigs killed at varying dates after experimental infection by feeding with culture of the *B. suipestifer*. It is there stated: " It is generally conceded that the proof of the ætiology of a disease consists of the isolation of the causative organism, its cultivation and identification on artificial media, production of the same disease by the introduction of cultures of such organism in normal subjects, followed by subsequent isolation and identification. These requirements have been met."

The sentences quoted are remarkable considering that they were made in the year 1928—remarkable because they constitute a repetition of the error which was made by Salmon, and subsequently by other investigators down to the discovery of the filterable virus, who failed to recognise that the disease experimentally produced by cultures was not swine fever since it was not contagious.

In a discussion on the subject (p. 1020) Dr. Biester said " this is a specific disease, just as specific as human typhoid fever, and we have a problem that in every way compares with it. An attack of the disease seems to be followed by an immunity . . . ."

Answering a question as to whether the disease was contagious, and whether attempted exposure had been tried, Dr. Biester said " It is an infectious disease. After these pigs had been fed a culture of virulent *S. suipestifer* they developed the disease. As to being contagious, it seems to be one of those diseases which, when one wants to produce it experimentally by contact, it cannot always be done. There seems to be a slight resistance or some aiding disease producing factor. A small amount of typhoid culture fed to a human being would produce disease, but in swine the condition produced by the *Salmonella suipestifer* seems to be more or less of a relative disease."

At the same meeting one of the speakers pointed out that the pictures which Dr. Biester had shown could be used to show the filterable virus lesions. Replying, Dr. Biester said: " We have had various typhoid and *so-called suipestifer* cultures, and we were unable to produce results with these, but the point is that all our studies were based upon herds where there was no evidence of hog cholera. We avoided that in every way so that there is

a proposition of *Salmonella suipestifer* infection which is not always associated with cholera. It can be absolutely independent of hog cholera."

After a careful study of the evidence provided in the article, it does not seem unfair to say that it is far short of proving that the disease from which the "field cases" were suffering was not hog cholera. Uncertainty must remain because the authors failed to do what would have removed all doubt, namely, to conduct inoculation experiments with a filtrate of blood of one of the dying field cases.

The age of these cases is against the view that they were suffering from paratyphoid infection—that is, infection with the *B. suipestifer*, because all authorities appear to agree that such infection does not occur naturally except in very young subjects, usually sucking or recently weaned animals, but in the field cases the pigs were from three to six months old, and in the two herds from which the diseased field cases were selected for study, the disease was so common that sick pigs could be obtained in quantities of four at a time.

On the other side it might be objected that, apparently in spite of opportunity, the disease did not spread to the healthy young pigs at the place where the experiments were being carried out, but that fact is in no way opposed to the view that the field disease was hog cholera. So far as the records show, the attempts to transmit the disease at the laboratory were not made with blood or other material taken direct from the field cases, but only with cultures from such material, which, as everyone knows, cannot have contained the virus.

An article by Hindmarsh and Edgar, of which an Abstract appears at a later page in this number of the Journal, purports to be an account of the isolation of *B. suipestifer* from cases of a contagious disease distinct from swine fever.

Without losing sight of any of the evidence in the article, we cannot agree that the possibility that the original disease in the abattoir pigs was swine fever was excluded. It is unfortunate that, as in the previous case, the authors failed to carry out the test that would have left no doubt on that point. One of the facts against the authors' main conclusion is that the pigs affected were not young animals.

## REVIEW.

**Index Veterinarius.** Imperial Bureau of Animal Health, Weybridge, Surrey. Annual Subscription £4.

The *Veterinary Bulletin* which was brought into existence by the Imperial Bureau of Animal Health in 1931 has been of great service to all those who desire to be kept in touch with current literature regarding animals' diseases, and the Index Veterinarius will be welcomed as a further help in the same direction. In it one may find, with a minimum amount of trouble, references to any recent contribution of note to the literature of veterinary science. The first volume (April, 1933) extends to 304 pages and is occupied by some thousands of references to articles published in any of the modern languages during the previous year. Both diseases and authors are indexed in alphabetical order, and the former are subdivided under the main heading, with the result that if one knows the name of either the author or the subject of the article both can be ascertained in a few moments. The list of journals from which the articles are indexed extends to 31 pages.

The volume reflects great credit on the editor and his assistants. The work is an ambitious and costly undertaking and we hope that it will have the success which it deserves.

# ABSTRACTS.

## SWINE FEVER.

By David and Schwarz.

The authors made observations and experiments on 286 animals infected with filtrates free from bacteria. After subcutaneous inoculation the onset of infection was shown on the second or third day by conjunctivitis and from the third to the fifth day by fever (over 40° C.), before any other signs were observed.

The authors state that very early, even after infection with pure virus, there occurs a secondary polybacterial infection, in which organisms of the paratyphoid and bi-polar kind play a special rôle. In the majority of virus-infected animals this secondary invasion occurs from about the seventh to the tenth day. In virus-infected animals without secondary infections (the pure virus disease) changes in the lymphatic glands are among the earliest and most striking. The earliest characteristic changes are, as a rule, so sparingly present during the first few days that the most searching examination is necessary to ensure that they are not overlooked. They do not appear regularly at definite places, but the lymphatic glands in the neck and at the entrance to the chest, and those of the rectum, spleen, kidneys, urinary bladder, stomach, large intestine, and the skin appear to be preferred sites. Hæmorrhagic and anæmic infarcts in the spleen are of great value for diagnosis, but while they are almost constantly present after artificial infection they are less frequent after natural infection. Diphtheroid lesions occur both in the pure virus disease and in consequence of the secondary bacterial infections. These lesions are most frequent in secondary infections with different paratyphoid bacteria, *and secondary infections may occur to an enzoötic extent in swine fever outbreaks.* [Italics not in the original.]

Hæmorrhagic-croupous pneumonia occurs both in the pure virus disease and in cases with a secondary bacterial infection. [This appears to imply that such a pneumonia may develop in the complete absence of bacteria.] A croupous pleuro-pneumonia with a tendency to necrosis caused by a bi-polar infection may prevail to an enzoötic extent in swine fever and in certain circumstances be mistaken for Schweineseuche (swine plague).

As a result of their observations regarding the pneumonia in Schweineseuche, the authors think that in every case in which the history and the lesions appear to point to an acute swine disease it is absolutely necessary to suspect Schweinepest, and to carry out the proper search for the virus of that disease, using all the known means that can be employed for the purpose. If that is not done there is a danger that the existence of swine fever may be overlooked, with consequent spread of the disease. (*Archiv. für wissen- und prakt. Tierheilk.* Vol. 62, 1930, pp. 315-343.)

## INVESTIGATIONS REGARDING SWINE FEVER AND PARATYPHOID INFECTION IN PIGS.

By R. Manninger.

In a previous article the author endeavoured, on the basis of his experience, which had extended over several years, to throw light on

the pathological changes in the independently developing suipestifer septicæmia (acute paratyphoid) of young swine. It was there pointed out that the most striking alteration in this disease is a hyperplastic swelling of the spleen, whereas formerly simple signs of a septic gastro-enteritis, with sparing hæmorrhages in the cortex of the kidneys, occasionally also under the epicardium and the pulmonary pleura or in the intestinal mucous membrane, as well as acute but never hæmorrhagic swelling of the lymphatic nodules, were found. The author's experience up to that point had permitted the conclusion that the cases of acute paratyphoid in the course of years had more and more been replaced by cases with a chronic character.

Subsequent experience has shown that the opinion then expressed requires qualification, at least for Hungary, inasmuch as the chronic cases with ulceration in the intestine are now quite in the background, whereas outbreaks of an acute character are so frequently observed that one would be justified in asserting that the acute paratyphoid, although possibly not the most important, is nevertheless one of the important diseases of young pigs. Thus, in the State Veterinary Hygienic Institute in 1929, 1930, and the first half of 1931, the disease was recognised respectively in 14, 36 and 25 herds, in which the cases of acute paratyphoid averaged 35, 28 and 25 per cent., respectively of the whole of the *post-mortem* material on pigs under six months old.

The author finds that recent experience also makes a further correction necessary, inasmuch as it has been found that, although the upper limit of susceptibility still appears to be six months, the most frequent age at which pigs are attacked is between two and four months; indeed in the West European breds two to three months is the age of the greatest susceptibility, and in the Mangalicaraffe breed, on the contrary, from three to four months.

The most important fact, however, from the diagnostic point of view is that, according to the experience of the last two years, in acute paratyphoid much more severe pathological changes occur than the author had observed up to 1925. In other words, it has been found that by no means rarely there are cases of acute paratyphoid in which there are such pronounced changes pointing to a hæmorrhagic diathesis that occasionally the lesions are absolutely indistinguishable from those of swine fever without croupous pneumonia and intestinal ulceration.

In such cases one finds, besides reddening of the skin and swelling of the spleen (to be described later), the known marks of hæmorrhagic diathesis, such as one is accustomed to regard as the most important characteristic of swine fever. Thus, apart from the fact that one finds blood extravasations up to the size of a penny in the gastric mucous membrane, and smaller, more numerous extravasations also in the intestinal mucous membrane, under the epicardium and the pleura, as well as in the renal cortex, it has to be emphasised that the different groups of lymphatic glands, especially those of the intestinal canal, are not only greatly enlarged, but, either throughout or in their periphery, show a speckled black-red appearance.

It is absolutely improbable that such cases of acute paratyphoid did not occur formerly, but such cases were probably diagnosed as swine fever, as was also done in cases without pronounced hæmorrhagic diathesis.

The reason for the renewed study of the acute paratyphoid disease

in young pigs was two-fold. First, there have been found cases in which the fatalities are confined to young pigs, whereas older animals kept in close contact with them have escaped although they previously had no opportunity to acquire immunity against swine fever.

On the other hand, the author has known herds in which swine fever, after it had occurred for years among the young pigs, was gradually brought to an end by inoculation with swine fever serum, but reappeared after some months, and then, in spite of a repeated employment of serum, further severe losses occurred. In such cases it is not right to assume that the first outbreak was actually swine fever, since in that case one would have expected that the disease, although under the control of immune serum, would have left a certain degree of active immunity, which ought to have kept the losses of the second outbreak within moderate limits even if the serum inoculation had not been repeated.

The author was able to prove by animal experiments in eight herds that the first outbreak was one of acute paratyphoid. A specially instructive case in which there was acute paratyphoid as an independent swine fever septicæmia was as follows :—

On a large establishment repeated deaths which could be ascribed to swine fever occurred among the young pigs (cross between Mangalica and Yorkshire) about four months old. This had happened every year since 1925. After the treatment of the entire stock of young pigs with swine fever serum the disease usually gradually came to an end without any serious loss. After some months there was a renewal of disease among the same animals which could be diagnosed as swine fever, and this, in spite of inoculation with serum, caused the death of from 20 to 50 per cent. of the animals. For example, in the year 1930 on account of the first outbreak on September 16th out of 261 pigs 11 died, whereas after the second outbreak on November 17th 52 of the animals died from the disease.

In the following year there was a group of 180 young pigs, weaned on January 2nd at the age of twelve weeks, in which two deaths occurred on January 17th. In the next few days four further deaths occurred, and the disease was diagnosed as swine fever; and on January 19th the inoculation of the whole of the young pigs with 20 to 30 c.c. of swine fever serum each was carried out. In spite of the inoculation there followed before the disappearance of the outbreak on February 28th, at intervals of from one to nine days, a total of 21 deaths among these animals. On January 25th, seven carcases in a fresh condition were sent to the Institute. This was done because of the facts which had occurred in the previous year, and the local circumstances made the existence of swine fever appear very doubtful.

In the whole of these carcases there was found besides hyperplastic spleen tumour, more or less intensive hæmorrhages, and also black-red discolouration of some of the greatly swollen lymphatic glands. Most striking were the changes in the carcase of one young pig whose organs and blood in the filtered and the unfiltered condition was used for an infection experiment in young pigs. In this animal the whole of the lymphatic glands were strikingly enlarged and very red, the retropharangeal, cervical, maxillary, precrural, gastric, mesenteric and bronchial glands were throughout or at some places of a black-red colour. There were lentil-sized hæmorrhages in the swollen mucous membrane, along with small loosely attached fibrinous deposits, and

also in the cortex of the kidneys many small hæmorrhages, which were also present in the cæcum and colon, as well as in the pleura of the lung in moderate number and somewhat larger. In the blood, spleen, and bone marrow the *B. suipestifer* was found in pure culture.

There were similar happenings two weeks later in another group of 148 young pigs, the progeny of animals in the same building. The first death occurred on January 30th, when they were about eight weeks old and not yet weaned, and were receiving grains in addition to the mother's milk. The carcases of this and three later dead animals were examined in the Institute and the lesions found were similar to those in the first group, and in all cases the *B. suipestifer* was found in the spleen in pure culture. As an example of the special severe lesions, the following is given :—

The cranial, cervical, pharyngeal, bronchial and precrural glands were swollen and very red; the stomach and mesenteric lymphatic glands were greatly swollen and discoloured black-red at their periphery or in specks. Numerous small hæmorrhages in the cortex of the kidneys, some up to bean size in the catarrhal reddened mucous membrane of the stomach, many lentil-sized hæmorrhages in the mucous membrane of the cæcum and colon, a few punctiform hæmorrhages under the epicardium, and hyperplastic swelling of the spleen.

In both cases the infection experiment gave a negative result, and the existence of swine fever could therefore be definitely excluded. Accordingly, no inoculation of the second group with swine fever antiserum was carried out, the diseased animals being simply isolated and lime compounds added to the diet to counteract rachitis, to which paratyphoid appeared to create susceptibility.

At the end of February this group appeared entirely sound, and rachitis was no longer recognised in it. The two groups were therefore now put together. Since, according to the experience of the previous years, one had to reckon with the possibility of an introduction of the swine fever virus at a later period, the combined herd, after further six weeks, during which the animals had developed well, were subjected to the simultaneous inoculation (serum and virus) against swine fever on April 18th. This was followed in the whole of the inoculated pigs by a well-marked reaction with loss of appetite and sometimes diarrhœa (a further proof that the previous outbreaks could not have been swine fever). In 27 of the animals the reaction took a serious character, but the pigs recovered with the exception of one which died in consequence of the reaction. The herd was then, after 24 days, on May 12th submitted to a further inoculation with swine fever virus by the method of Hegyeli, which caused no clinically recognisable reaction.

The author maintains that the above-described facts brought the strict proof that " acute independent paratyphoid of young pigs can actually occur under the anatomical picture of a pronounced hæmorrhagic diathesis with black-red discolouration of the lymphatic glands, such as one has hitherto considered characteristic of swine fever."

Similarly, through the negative result of inoculation with filtrates of organs and blood into susceptible swine in seven other cases with similar *post-mortem* findings, the presence of *suipestifer* bacilli in the spleen and blood, and the absence of filterable swine fever virus was proved, and it had thus to be recognised that in these cases one had to do exclusively with acute paratyphoid which, partly owing to insuffi-

cient diet (rachitis or osteoporosis) and partly through cold, had taken on the picture of a pronounced hæmorrhagic diathesis.

In all these cases the infection experiments were made on young pigs of the English breed (Yorkshire) weighing from 20 to 35 kg., and in many of the experiments two to four (and altogether 28) animals were used. To ensure the absence of swine fever infection the experimental animals were strictly isolated under close observation for some days, and in each experiment one or two animals were left uninoculated as controls.

Emphasis is laid on the fact that in each experiment at least one pig was inoculated with unfiltered diseased material, which therefore contained *suipestifer* bacilli, in order to guard against the suggestion that during the filtration of the infective material the swine fever virus had been lost. The experiments with unfiltered material had shown an interesting result, since here, in contradiction to the parallel experiments with germ-free filtrates, the animals, without exception, became more or less diseased, and temporarily had *suipestifer* bacilli in their blood, except in one animal which recovered.

"In view of the difficulties which exist in appreciating the *post-mortem* findings in distinguishing between swine fever and acute paratyphoid of young pigs, the usefulness of bacteriological examination has to be considered. Unfortunately the difficulties in the diseases of pigs of the age under six months, which are specially considered in this article, cannot be put completely aside, since *suipestifer* (paratyphoid) septicæmia very frequently occurs in connection with swine fever as a complication, especially in young pigs. Thus, in the last year and a half, in the carcases of young pigs in which one had to assume the existence of swine fever, we found *suipestifer* bacilli in 16 of 32 cases, that is, half of the cases. Hence, the detection of *suipestifer* bacilli in the blood, spleen, or other organs, has by itself, no importance with regard to the distinction between swine fever and acute paratyphoid."

"On the other hand, the recognition of *suipestifer* bacilli has great importance in diseased pigs over six months old, since here the existence of *suipestifer* septicæmia speaks decidedly for swine fever, because in our experience in animals of this age and over six months an independent *suipestifer* septicæmia never occurs, but always arises as a complication of swine fever."

Certainly in adult swine one meets with acute *suipestifer* attacks in consequence of swine fever less frequently than in young pigs; at least in our *post-mortem* records we have been able to diagnose *suipestifer septicæmia* only in 25 per cent. of the cases of swine fever in adult animals.

When, however, the *suipestifer* bacillus is recognised in the organs of old animals the discovery is welcome.

The diagnosis of swine fever in the animals under six months old, as compared with the diagnosis in older animals, is also made more difficult by the fact that in young pigs croupous pneumonia and characteristic (button) ulcers in the cæcum and rectum occur much more rarely than in the course of swine fever in adult animals, apparently partly because in young animals the disease runs a quicker course than in old ones. Thus we have hitherto observed croupous pneumonia or intestinal ulcers, or both of these lesions, in 35 per cent. of the adult animals as against only 9 per cent. in cases in young pigs.

If one had to consider the detection of *suipestifer* bacilli, or of croupous pneumonia and intestinal ulcers, or other changes which raise a suspicion of swine fever in adult animals, as sufficient for the definite diagnosis of swine fever, our material would have allowed the diagnosis of swine fever to be made without any hesitation in 52 per cent. of the cases, and in only 48 per cent. would one have had to rely on other lesions or on the particular existing circumstances.

On the other hand, with regard to the occurrence of swine fever in young animals, it was possible only in 9 per cent. of the cases to make a certain diagnosis simply on the ground of the evidence for swine fever afforded by the changes in the lungs and intestines. In the other cases the diagnosis would have had to be in part confirmed by taking into account the existing circumstances, as for example, the recognition of simultaneous disease in the older animals in the same herd. (*Deutsche tier. Wochens,* Vol. xl, 1932, pp. 113-120.)

## SOME OBSERVATIONS ON THE *B. SUIPESTIFER* SEPTICÆMIA OF PIGS.

By W. L. Hindmarsh and G. Edgar.

The authors state that the fact that *B. suipestifer* had been proved capable of causing a septicæmia as well as enteritis was to a large extent lost sight of when swine fever was found to be due to a virus, but later experimental work brought to light that *B. suipestifer* could be primarily the cause of necrotic enteritis, apart altogether from its association with the virus of swine fever, and it was also shown that pigs, especially young stock, might die from septicæmia due to infection with *B. suipestifer* before necrotic lesions developed to any extent in the bowel. That it might be the cause of septicæmia in older pigs, and that pigs might recover without showing symptoms of necrotic enteritis, and that such septicæmia might readily be spread by contact, was not generally recognised, although references to acute paratyphoid of young pigs occur from time to time in veterinary literature. Most authors are of opinion that predisposing conditions play some part in creating susceptibility to infection, and it has also been suggested that *B. suipestifer* may acquire a virulence, hitherto lacking, by passing through the pig. The authors note that, although there are various records of human infection with *B. suipestifer,* the infection has rarely been traced to pork as its source.

The subject was brought under the notice of the authors by the fact that the condemnation of pigs on account of septicæmia at Homebush abattoirs was assuming considerable proportions, and it was desired to ascertain the cause of the condition.

The lesions on which septicæmia had been diagnosed by the inspectors at the abattoirs in nine cases included congestion of the spleen, petechiation of the kidneys, congestion of the lungs (pneumonia in one case), reddening of the skin, hæmorrhages in the lymphatic glands, including those of the intestine, congestion of the small bowel (one case), and necrotic enteritis (one case). The most constant lesions were petechiation of the kidneys and reddening of the skin.

The authors observe that previous investigators had recorded that in acute *B. suipestifer* infection swelling of the spleen is an almost constant lesion.

Bacterial investigation of the specimens received from nine cases showed that a Gram-negative bacillus, not fermenting lactose, could be readily recovered except in one pig. The organs from which this bacillus was cultivated included the spleen, liver, kidney, lung and gastric lymphatic glands.

In view of the fact that the organism appeared to be one of the paratyphoid group, it was decided to examine bacteriologically material from pigs: (*a*) which had been condemned by the inspector as unfit for food on account of lesions other than those indicative of septicæmia; (*b*) which had been passed as fit for human consumption. As a result the bacillus was found in 17 out of 27 cases.

Summarising the results it was found that not only had a paratyphoid bacillus been isolated from the carcases of pigs condemned on account of septicæmia, as shown in the first examination, but also from animals condemned on account of septic pneumonia, and even from 14 of 18 carcases passed for consumption a similar organism was cultivated.

In view of these findings it was decided, (*a*) to test the various reactions of the organism recovered from the different groups of pigs, so as to identify it; (*b*) to test the pathogenicity of the organisms for pigs, and its infectivity by cohabitation of infected and non-infected pigs.

The fermentation reactions of the organism were found to be typical of a *B. suipestifer.*

*Inoculation Experiments.*—In view of the fact that swine fever had been present in this state some years previously, it was considered necessary to eliminate a possibility of the lesions produced in experimental animals being due to this disease and to show that the bacillus isolated was capable of causing lesions similar to those in the pigs condemned at the abattoirs.

Infection experiments were therefore carried out with, (1) cultures which had been passed through a rabbit and (2) cultures which had been subjected to repeated cultivation.

*Cultures which had been Passed Through a Rabbit.*—A pig inoculated with broth culture of *B. suipestifer* which had been recovered from an experimentally infected rabbit showed marked elevation of temperature, suppressed appetite, and disinclination to move after 24 hours. On the third day following inoculation the animal, while still obviously ill, was killed. Beyond a slight hæmorrhagic condition of some of the lymphatic glands no gross lesions were detected *post-mortem. B. suipestifer,* however, was recovered from the heart blood, liver, spleen, and kidneys.

*Cultures Repeatedly Cultivated.*—Two pigs were inoculated, one with 5 c.c., and the second with 1·5 c.c., of a broth culture of *B. suipestifer* which had been sub-cultivated several times. The animals were isolated separately and daily observations were conducted. After 24 hours both pigs showed a marked rise in temperature, which was followed on the second day by suppression of appetite, diarrhœa and loss of condition. Those conditions persisted for four days, when the pig which had received the smaller dose of culture was killed, six days after inoculation.

In this and all the other cases the carcases were scraped in hot water to reveal the presence of any skin lesions, and in this pig the

skin was of a bright scarlet colour around the snout, on the ears, along the ventral surface of the body, on the fore legs from the feet to the elbows, and on the hind legs from the feet to the hams. The carcase was quite emaciated and internally there was some congestion of the gastric mucosa and necrotic enteritis of the small intestine and cæcum. The kidneys were congested with a few petechiæ and some ecchymotic areas of similar lesions were present in the liver and lungs. The *B. suipestifer* was recovered from the blood, liver, spleen and kidneys. The pig which had been inoculated with 5 c.c. of culture showed an elevation of temperature, diarrhœa and general malaise for six days following the inoculation. On the seventh day the temperature had fallen considerably, and on the eighth day was normal, the diarrhœa had ceased and the animal appeared to be in good health. On the seventeenth day following inoculation this pig was killed, at a time when it appeared in every way healthy. No gross lesions were detected on *post-mortem* examination and *B. suipestifer* was recovered from the kidneys only.

The authors concluded from these experiments that *B. suipestifer* is capable of causing a severe systemic disturbance of a septicæmic nature in pigs. They say that while this had been noted by workers in the United States of America and Europe the results were there obscured by the fact that swine fever is enzoötic in those countries.

Gilruth* in New Zealand, however, showed definitely that *B. suipestifer* could of itself produce septicæmia, and that was confirmed by the authors' findings.

The next consideration in connection with the pathogenicity of *B. suipestifer* was to determine whether the condition could be readily transmitted from an infected pig to healthy pigs by co-habitation, under hygienic conditions. In these experiments it became necessary to eliminate the possibility that the pigs intended for experiment already carried the infection. This was done by submitting sera from all pigs used to the agglutination test against the strain of *B. suipestifer* used. In all cases the pigs gave a negative reaction prior to being used in the experiment.

In the first experiment a pig which had been inoculated with a culture of *B. suipestifer* was placed in contact with a healthy pig. The animals were placed in concrete isolation boxes which had been disinfected prior to use. The inoculated animal showed rise in temperature and rather profuse diarrhœa 48 hours after inoculation, while symptoms did not appear in the in-contact pig until the seventh day after being placed in contact, when it showed elevation of temperature, total suppression of appetite, and diarrhœa. Remissions and exacerbations of temperature took place over a period of seven days, after which both pigs completely recovered. Subsequent agglutination tests with sera from these pigs against *B. suipestifer* were strongly positive.

It was thus concluded that even when pigs are kept under hygienic conditions the infection can be readily transmitted from infected to healthy pigs by contact.

*Infection by Ingestion.*—A pig was drenched with 5 c.c. of a 24 hour broth culture. After 48 hours temperature had risen 3° F., and there was suppression of appetite and general malaise. On the third day there was profuse diarrhœa. The diarrhœa persisted, and condition

* Annual Report, Chief Veterinarian, Dept. of Agriculture, New Zealand, 1898-9.

was rapidly lost until the thirteenth day after drenching, when the animal died. *Post-mortem* examination revealed extensive lesions of necrotic enteritis. *B. suipestifer* was recovered from the liver, but not from the blood and kidneys.

A pig was fed 10 c.c. of 24 hour broth culture mixed with pollard. Within 48 hours there was a rise of 4° F. in the temperature, loss of appetite, and the pig was obviously ill. On the following day the animal was affected with acute diarrhœa. During the following three days the temperature fell, and the condition of the fæces improved. The animal subsequently recovered completely.

*Lesions Found in Pigs Subject to Experiment.*—Of the seven pigs, subjects of the experiment, four were inoculated with culture, one placed in contact with the inoculated pig and two were infected by mouth. Three of the four inoculated animals were killed, one infected by the mouth died and the remaining three recovered.

A comparison of the lesions seen in the pigs on *post-mortem* examination is of interest. The pig killed three days after inoculation showed only a hæmorrhagic condition of the lymphatic glands; that killed six days after inoculation showed petechiation of the kidneys, liver and lung, reddening of the skin and necrotic enteritis, whilst that killed 17 days after inoculation showed no gross changes.

From the first mentioned pigs, *B. suipestifer* was readily cultivated from various organs, whilst in the last case this organism was recovered from the kidneys only. This throws some light upon the results of the examination of organs of pigs which were passed for human consumption. In the incubative stage and in the stage of recovery the carcases of pigs may be passed for human consumption although *B. suipestifer* is present in the organs and blood.

Of the pigs fed with culture, one succumbed and on *post-mortem* examination showed extensive lesions of necrotic enteritis, but no gross lesions in other organs. It appears, therefore, that necrotic enteritis may be caused by this organism when the pigs are infected *per os*.

### Summary.

These experiments indicate that *B. suipestifer* is an organism which is capable of causing considerable mortality in pigs, and that it may spread readily amongst pigs in contact, even when the animals are kept under hygienic conditions. (*Veterinary Research Report, No.* 6, *Dept. of Agric., New South Wales.*)

## THE DIAGNOSIS OF SWINE FEVER.

By O. Waldmann.

In a previous article which appeared two years earlier, the author submitted a report on the diagnosis of swine fever. He there described the clinical signs, and also the pathological lesions of acute and hyper-acute cases, that is to say, not complicated by secondary infections. He was able to show, on the basis of a very large series of experiments, in what proportion the different organs are attacked, and what is the delay required for the development of the lesions. He also there dealt with the degree to which swine fever prepares the ground for secondary bacteriological infections, and stated that already on the eleventh day

after inoculation, or the sixth or seventh day after the appearance of the fever indicating infection, in round figures, 80 per cent. of all the animals infected by virus without bacteria present secondary infections.

Since the publication of that report a series of experimental and histological researches carried out in America, France and Germany, have furnished some interesting and important opinions on the pathogenesis of swine fever, and on the diagnosis and prevention of the disease. In his previous report the author showed that the lesions of acute pure swine fever are those of a hæmorrhagic septicæmia. The hæmorrhages are the most obvious of the macroscopic lesions. They indicate a preponderating lesion of the blood vascular system. They are met with in all the organs, are more or less extensive according to the structure of the organ, and occur independently of structural alterations in the capillaries.

On the other hand, on histological examination one finds in the pre-capillary and post-capillary veins some very marked lesions of a progressive or regressive nature, which in the different organs are accompanied by parenchymatous lesions of an entirely different kind.

For example, the blood lesions provoked in the lymphatic glands by the virus of swine fever are well known. They are caused by hæmorrhages in the lymphatic parenchyma and lymphogenous resorption of erythrocytes; but the author attributes decisive importance to the histological vascular lesions found in the lymphatic glands. In these there is an increase in the number of endothelial or adventitious cells, or, in lesions already in a state of regression, hyaline degeneration, thrombosis, or necrosis of the walls of the vessels.

In the typical lesions of the kidneys, these organs are often astonishingly pale and present, especially in the cortical layer, numerous hæmorrhages of the size of a pin's head. The glomeruli, contrary to what is seen in swine erysipelas, do not show hæmorrhages. The lesions of the vessels are important in the kidney; spasmodic contractions of the arteries produce decolouration of the organs and hyaline degeneration of the inner wall of the vessels, which may be accompanied by cloudy swelling and hyaline degeneration.

In the urinary apparatus the bladder is also a predilection seat of hæmorrhages. One there finds foci varying in size from a pin-head up to the formation of a hæmatoma. As histological examination shows, these hæmorrhages come from the sub-epithelial capillary net-work.

The pronounced intestinal lesions in swine fever do not present any special histological characters. When one finds in the spleen anæmic and mixed infarcts they proceed from hyaline necrosis and thrombosis of the follicular arteries. The lesions of the central nervous system, described almost simultaneously but independently by Seifried in the United States, by Dide in France, and by Röhrer in Germany, have a special importance. In the central nervous system one has observed microscopically only some very rare cases of hæmorrhages in the brain and under the dura mater; on the contrary, in acute swine fever one finds histologically a diffuse non-purulent encephalomyelitis. Seifried, who attempted to make a classification of the inflammations provoked in the central nervous system by different kinds of virus, pointed out the existence of a group of neurotropic viruses, such as of rabies and Borna disease, as opposed to a group of viruses which are at the same time organotropic and neurotropic. In the second group, along with

very variable organic lesions, we find lesions of the central nervous system, which, in principle, are of the same kind and have the same localisation. To this category belong, among the diseases of animals, distemper of the dog, fowl plague, and swine fever. In this group the principal lesions are vascular, with in the second place lesions of the ganglionic cells. In swine fever the most marked lesion is that of the vessels.

The vessels themselves show swelling of the endothelium, a cellular infiltration of the outer coat of the vessel wall, and, finally, collections of cells in the perivascular lymphatic spaces. The vessels which in their normal histological condition one hardly perceive stand out clearly in the form of cellular cords of varying thickness. The lesions have no predilection seat in the central nervous system. They are found in equal numbers in the grey substance and in the white, but are most numerous in the two prolongations of the corpus callosum, and in the superficial parts (immediately under the meninges), and internally (in the neighbourhood of the ventricles).

The lesions in the nerve cells are not very striking, and, in the author's opinion, they have only a secondary importance.

The same lesions were found in the spinal cord both in the grey substance and in the white. The lumbar part of the cord is specially attacked.

*Diagnosis.*

In the present state of knowledge regarding the pathogenesis of swine fever, it ought not to be difficult to arrive at a diagnosis by clinical and *post-mortem* examination in those cases in which the virulence of the virus is as pronounced as the signs of acute infection, that is in outbreaks spreading rapidly in the herd and attacking animals of all ages. But anyone who has had long experience in connection with swine fever knows that the virulence may vary greatly, *and that a weak virus may occasionally cause in single animals or in a herd, forms of disease or epidemic types which cause the greatest difficulties in diagnosis.*

Everyone knows breeding herds in which, in spite of the quality of the animals, the business is not successful. The oldest animals appear healthy, but the losses among the young pigs, which at first did not exceed 5 to 10 per cent., rise to 20 or 40 per cent. Few deaths occur among the old animals. The symptoms in the young pigs, three or four weeks old, are diarrhœa, cough, catarrh of the mucous membranes, and cutaneous eruptions. Those that do not die become typically unthrifty. Some of them recover, but usually remain a little inferior to the normal condition of development. At the *post-mortem* examination of individual animals in the herd one hardly ever finds what one expects in acute swine fever. As a rule, medicinal treatment fails, and changes in hygiene and in feeding produce only a temporary improvement. This state of affairs may persist in a herd for years. During the summer the losses remain moderate, but they constantly increase in winter. There can be no question of any profit from herds of that kind.

In such conditions one often calls in the aid of the nearest Research Laboratory, to which one sends diseased young pigs or carcases. Here also the *post-mortem* examinations reveal extremely variable lesions, and in many cases they are so slight that they cannot be brought into relationship with the severity of the disease.

Very often a searching bacteriological examination reveals the presence of some member of the paratyphoid group, *B. suisepticus*, streptococci, *B. coli*, etc., which are capable of causing septicæmic conditions. But the fact that in a herd one finds different bacteria in different carcases, demonstrates that it would be a mistake to come to the conclusion that the disease was the result of a bacillary infection, and nothing is more characteristic of our helpless condition with regard to diagnosis than these cases in which material sent at the same time to different laboratories brings back a different diagnosis from each.

The attempts to recognise the cause of the disease in herds attacked in this way were first made in recent years by Nusshag and Pröscholdt, and during the past three years by the present author with the collaboration of David and Röhrer. The idea that swine fever plays a rôle in such herds here presented itself in cases in which it was reported that disease had prevailed in the acute form for months or years previously.

What are the means, clinical or *post-mortem*, available for the diagnosis of swine fever? One knows from the writing of the old masters, Dorset, Hutyra, Uhlenhuth, and others, the great importance of the experimental transmission of the disease. Only pigs can be used for that purpose.

The experimental transmission of the disease is not easy. The choice of the animals to be inoculated at the outset presents some difficulties. One ought to have, in every respect, the certainty that the animals come from absolutely healthy herds. It is not sufficient to inspect the herd before selecting the animals; these must afterwards be submitted to a prolonged observation for several weeks in quarantine at a distance from the establishment. An essential condition of success is the strict isolation of the animals that are to be inoculated. For this purpose the author had constructed boxes which could be sterilised by steam before the experiment in a steriliser specially made for the purpose, and which are placed in special buildings separate from the Institute. They are provided with an automatic arrangement which prevents any contact of the animal with utensils used in feeding during the whole course of the experiment. The material to be used for inoculation ought to be taken from carcases as fresh as possible on account of the fact that natural putrefaction, which proceeds very rapidly in carcases and organs sent during the warm season, alters the virus and may destroy it completely, and even in cases of acute infection make the results of the experiment doubtful or entirely negative.

The inoculation with germ-free filtrate is made under the skin in a dose of not less than 10 c.c. Observation lasts for 21 days. No temperatures are taken during that time because experience has shown that the daily taking of temperatures in a central research station, even with the most minute precautions, may result in accidental infection with swine fever. Two animals are used for each experiment.

The following is given as an example of the result of an experiment:—

If blood is taken on the third or the fourth day of fever in a pig experimentally inoculated with highly virulent material and inoculated to a healthy young pig, one obtains in nearly 100 per cent. of cases a positive result, with an acute course and a fatal termination within the period of observation of 21 days. But if it is a case with a subacute or chronic evolution the result of the attempt to transmit the

disease by experiment becomes more uncertain in proportion to the time that has elapsed since the infection.

In the course of the experiments it has been observed : The inoculated animals are visibly ill but they do not die. Often they recover completely, and at the end of the experiment one observes in them only a very feeble difference in weight compared with the controls. In many cases this slight difference of weight constitutes the only objective symptom in the animals used. In order to determine whether an infection of these animals has been realised it was indicated to practise quite early a reinfection with a very active virus, but it was found that the subjects which had presented signs of the disease and afterwards recovered were refractory. But a new difficulty occurred : it was known that a hyperactive virus may break down the immunity conferred by a feeble virus. On that account one of the surviving animals is now sacrificed for the purpose of the experiment and the other is submitted to a reinfection. However, even with this procedure there remain a good many doubtful or undetermined cases.

It must be inferred from these facts that the experimental transmission of the disease is a difficult method. We have in consequence, been a little disappointed with regard to its practical value for diagnosis. We have received in all about 200 specimens from living or dead animals or parts of diseased organs from herds suspected of swine fever, and in 154 of these the experimental transmission of the disease was attempted. In 20 cases the material was derived from imported animals or their organs ; in 134 cases they were from herds suspected of acute swine fever (68) or chronic swine fever (66).

Out of the 68 cases of suspected acute swine fever we found that disease had to be excluded in 31 cases in which the animal experiment was negative and the researches conducted in the herd and in the laboratory indicated other causes (defective housing, bad feeding, poisoning, swine erysipelas, etc.). In 15 other cases it was not possible to decide with certainty. The result of the transmission experiments (six times negative, once doubtful, and eight times positive) for various extrinsic reasons could not be confirmed by observing the evolution of the disease in the herds in question. The herds were in part disposed of, or the owners withdrew their assistance. These 15 cases had therefore to be struck out from consideration in regard to the value of control by experimental transmission of the disease.

On the other hand, the 22 remaining herds suspected of swine fever are important with regard to this question. In these cases the existence of swine fever was actually demonstrated beyond any doubt by the experimental transmission test. In four of them the lesions were sufficient to establish the diagnosis. The result of the transmission test was negative in six cases and doubtful in four ; *but in the 22 cases the evolution of the disease in the herd and the subsequent examinations demonstrated the existence of swine fever.* The attempt to diagnose the disease by experimental transmission thus failed in 45 per cent. of the cases.

The investigations in the suspected cases of chronic swine fever had similar results. In 26 cases the infection experiments were undeniably negative. The diseases which could be recognised in the majority of the young pigs were : swine erysipelas, insidious broncho-pneumonia, defective hygiene, and the presence in the same herds of different bacterial infections, parasitic diseases, avitaminosis, etc.

In 14 cases—with seven negative, three doubtful, and four positive results—for various reasons it was not possible to arrive at a definite diagnosis by later experiments, so that these cases had to be eliminated from further consideration.

In 26 out of 66 cases the suspicion of swine fever was confirmed by the subsequent course of the disease and by practical examinations conducted in the herd. These were undoubtedly cases of the virus disease (swine fever). The result was negative in twelve cases (46 per cent.), doubtful in one case, and positive in 13 cases (50 per cent.).

As a result of these very laborious researches regarding the diagnostic value of animal inoculation, the author had to conclude that its employment in herds infected with the acute or chronic form of the disease did not yield positive results in more than 50 per cent. of the cases. The long period required for the test renders it unsuitable in cases of suspected acute swine fever, because the progress of the contagion in the herd often gives a quicker result than animal experiment. In the chronic cases also the method is inadequate. Since only the positive result of an inoculation has a decisive value it has to be several times repeated with infective material coming from different animals of the same herd at different times, but such repeated examinations could not be carried out without State assistance, which is only rarely obtainable, *e.g.*, in very large breeding herds of great value. One must, therefore, endeavour to obtain other methods of obtaining certainty in the diagnosis of swine fever.

It is well known that in a large number of the diseases provoked by ultra-visible viruses a certain diagnosis can be founded on the histology of the lesions; rabies, Borna disease, fowl plague, louping-ill, human epidemic poliomyelitis and encephalitis.

The final conclusions drawn by the author are as follows:—

1. In our opinion the diagnosis of chronic swine fever in a herd is justified when after several *post-mortem* examinations with doubtful results the inoculation test is positive, that is when the inoculated animals die or, if they survive, are found to be undoubtedly immune.

2. The existence of swine fever is contra-indicated when in at least two animals the results of the *post-mortem* examination have been doubtful, the histological lesions of the disease are absent, and the inoculation test has had a negative result.

3. In cases in which, after one or several examinations with doubtful results, an inoculation test has had negative results, there are found histological lesions of a doubtful character in several organs, the proper course is to repeat the examinations with a view to obtaining a certain diagnosis.

The article is concluded as follows: The reader perhaps knows, that, especially in German literature, one often encounters examples of chronic infection in large herds—continuing for years, characterised by pretty serious losses at times, and that with or without evidence of acute swine fever (*Viruspest*). Various authors have proved the certain existence of the disease in such herds, but the author observes that demonstration of the presence of the virus in herds of that kind is very difficult to obtain by the means at our disposal. Success depends less on the ability of the experimenter or the precautions he has taken than on the conditions, still quite unknown, which permit the persistence of the virus in animals that have recovered from an acute infection, with or without being subsequently unhealthy in appearance. It is not

now denied that such animals may be carriers of the disease, but we still know very little as to the proportion of the carriers and the form and the duration of the excretion of the virus. In the author's own examinations of seven " wasters "—the residue of numerous experiments with virulent material free from bacteria—he was able to demonstrate the presence of the virus in only three of them, in one on the thirty-first day after infection, in the second on the forty-first day, and in the third on the hundred and ninety-eighth day. This agrees approximately with the results registered in the infected herds. By the methods available we cannot expect a positive result in more than 50 per cent. of the diseased animals.

In these conditions the adoption of histological examination for diagnosis may prove of some service. (*Office International des Epizooties*, *Bulletin* 1, Tome vi, May-June, 1932, pp. 41-58.)